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# PLANT PHYSIOLOGY

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## ERRATA

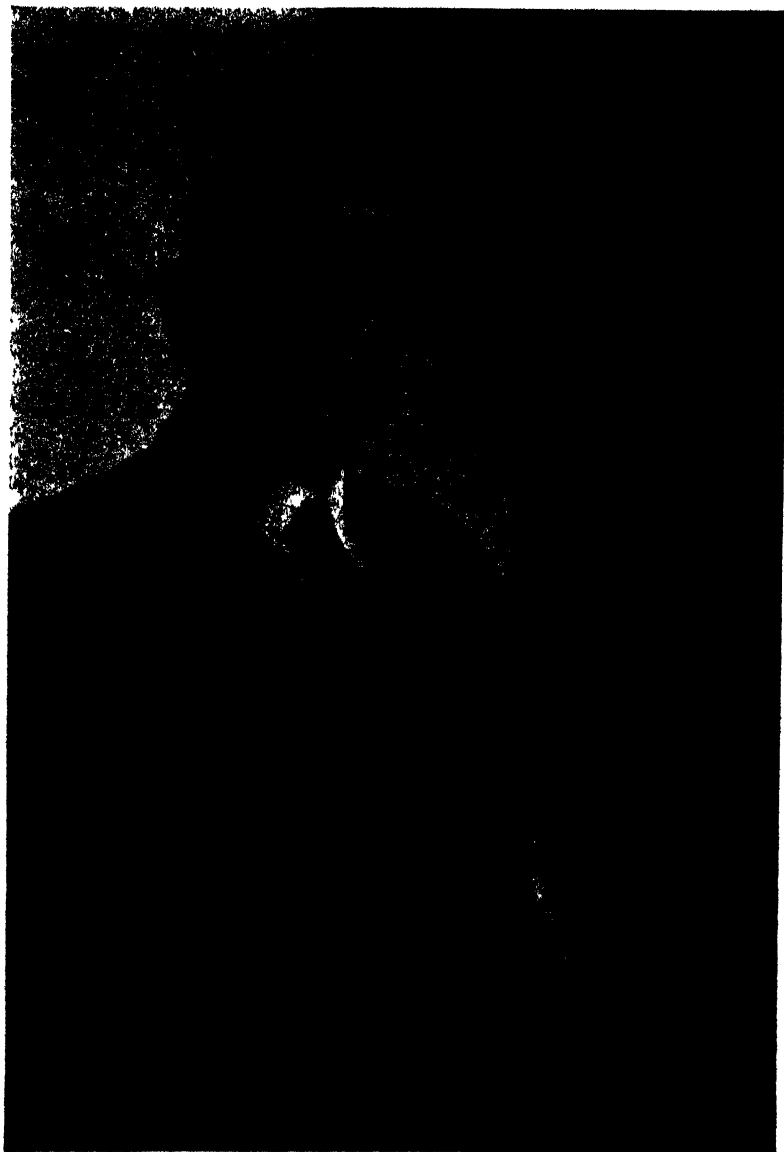
### VOLUME 12

- Page 3, fifth line above footnote, for "glass" read gases.
- Page 54, figure 1, for "M.P.R." (occurring twice) read M.P.H.
- Page 58, line eight from bottom, for "SCHNADER" read SCHANDER.
- Page 65, line 15 from bottom, for "(19)" read (20).
- Page 66, line one, for "with" read which.
- Page 74, line 9, for "HALL" read HALE.
- Page 77, citation 17, for "51" read 15.  
    citation 20, for "Protoplasm" read Protoplasma.  
    citation 26, for "395" read 305.  
    citation 27, for "TUTTLE, G. U." read TUTTLE, G. M.
- Page 80 dele line 2, and insert instead: "of microchemical color tests have been proposed.  
    HANSEN (23) describes"
- Page 138, line 2, for "0.50-ml." read 0.05-ml.
- Page 205, citation 7, for "the Delicious apple" read apple trees.
- Page 412, line ten, dele reference (OKUNUKI, 61).
- Page 418, fig. 1, switch at far left in S should be double-pole, double throw, not a reversing switch.
- Page 500, line six, paragraph on experimentation, insert "no" after word caused.
- Page 503, line two from bottom, for "and that" read *i.e.*
- Page 777, line twenty-one, dele hyphen.
- Page 793, line twenty-six, period at end of line.  
    line twenty-seven, for "although" read Although, and insert comma at end of line.  
    line twenty-eight, for "This" read this.  
    last line, dele "ing" at beginning of line.





**THIS NUMBER OF PLANT PHYSIOLOGY**  
**IS DEDICATED TO**  
**RODNEY HOWARD TRUE**  
**IN CELEBRATION OF**  
**THE SEVENTIETH ANNIVERSARY OF HIS BIRTH**  
**OCTOBER 14, 1866**



RODNEY HOWARD TRUE

OCTOBER 14, 1866



# PLANT PHYSIOLOGY

JANUARY, 1937

## DISTRIBUTION OF THE VELOCITIES OF ABSORPTION OF WATER IN THE ONION ROOT

HILDA F. ROSENE

(WITH FIVE FIGURES)

Although many investigations have been made there is, up to the present, no available study which furnishes reliable quantitative data on the distribution of the rates of water absorption in any one root. Only recently (1, 8) has there been any attempt to make the investigations strictly quantitative in character but all studies are open to adverse criticism since none were carried out under adequately controlled conditions. It appears that the only method at present available which provides for complete control of conditions using the *intact, uninjured, and unstimulated* root is that used in the present study.

Since reviews have been presented by other investigators (1, 4, 8, 9) no comprehensive survey of the literature will be attempted. It is important, however, to call attention to certain inadequacies of the methods employed by the more recent investigators. POPESCO (4) made determinations of the rates of water absorption on single roots of a number of different plants, but his results were only relatively quantitative. He employed both direct and indirect methods which involved: (1) the isolation of given regions of a root by covering these with cocoa-butter and then placing the root in a U-tube, one arm of which, drawn out to a capillary, was graduated in arbitrary units; (2) the use of dyes and microchemical reagents; and (3) plasmolysis. HÖHN (1) made unsuccessful attempts to use a modified form of POPESCO's "cocoa-butter" procedure. He was unable to obtain a perfect seal with rings of cocoa-butter and he points out the impossibility of employing inelastic material to cover the region of elongation. To cover various regions of the root HÖHN substituted oil for cocoa-butter. His experiments were carefully carried out under known conditions of temperature and humidity. The chief criticism of his work is given by SIERP and

BREWIG (8, p. 115) who show that HÖHN's conclusions regarding the distribution of the rates of water absorption in a single root are not valid because they are based on comparative measurements of (1) different roots, and (2), on the same root, but at different time periods. Furthermore, as will be shown later, HÖHN's tabulated results do not warrant the conclusions that he gives.

URSPRUNG and BLUM (9), HÖHN (1), and SIERP and BREWIG (8) call attention to the limitations of all methods involving dyes and microchemical reagents, pointing out that it is impossible to determine the rates of water absorption when quantitative relations between the spread of the dyes and the movement of the water are not known. On this basis the results of POPESCO (4) and KELLER (2) employing dyes may be questioned. URSPRUNG and BLUM (9) based their conclusions on suction-pressure measurements. As pointed out by SIERP and BREWIG (8, p. 115) their method is limited because such measurements could be evaluated quantitatively only if the suction values remained constant when the root absorbed water and it has not been established that such a relation exists.

SIERP and BREWIG (8) used a number of micropotometers making simultaneous measurements on the same root. They state that so far as they know this was the first time such a procedure had been followed. However, the method employed in the present investigation, which also involved simultaneous measurements by several micropotometers on the same root, was first reported by ROSENE and LUND in 1934 (6). The technique followed by SIERP and BREWIG has serious limitations. They made measurements on the roots of plants which were in an inverted position with respect to gravity, thus producing conditions of stimulation. In order to insure water-tight connections they wound thread impregnated with wool fat around the root. They maintain that after the experiment no injury to the root was apparent but they do not give the criteria or tests upon which their conclusion was based. Wrapping the roots with thread in this manner would produce mechanical stimulation and would interfere with respiratory activities. The quantitative determinations made by HÖHN, and by SIERP and BREWIG, were expressed in milligrams per linear length, assuming that the diameter of the root was uniform throughout its length. It is obvious that such a technique furnishes data which is only relatively quantitative. Of far greater value are reliable data on the rate of absorption per unit of surface area.

Determination of the distribution of the velocities of absorption of water in roots should therefore be made on intact, uninjured, unstimulated roots, and under conditions which permit careful control of such conditions as temperature, light, oxygen, and humidity. It should be possible to make simultaneous measurements of water absorption by different regions, and

to determine the rates of absorption per unit of surface area. It is believed that the present technique fulfills these requirements.

### Apparatus and method

The experiments were conducted at room temperature (25° C.) in a basement room, the temperature of which did not vary more than 2° during the longest experiments, which were of several days' duration. The observations were made on the roots of *Allium cepa*, which for the most part were grown either in tap water, or in a saturated atmosphere in vessels through which air was continuously passed.<sup>1</sup> In certain experiments, observations were made on roots which had developed when a given onion was growing in the experimental apparatus. Several experimental chambers were constructed, making it possible to run controls while varying the conditions in one or more chambers.

The details of one experimental chamber are shown in figure 1. The removable glass cover (A, fig. 1) rests in a groove (F) in the transite base (B) which is attached to a rack and pinion stand providing vertical adjustment for the base and cover. The base has four perforations lined with bakelite tubing through which connections pass from the interior to the exterior of the chamber. The onion bulb rests upon a cork ring which is placed in a hole in a swivel attachment (C). The swivel attachment is supported by the glass tube (G), which is cemented to a brass rod (D). The brass rod passes through a perforation and is supported on the outside by a three-way mechanical micromanipulator. With this arrangement the onion bulb may be moved to any desired position by manipulation from the outside. Each micropotometer (H) consists of a calibrated glass tube made from carefully selected millimeter pyrex tubing of uniform bore. One end of each tube (a, b, c, d, fig. 1) was ground down on two opposite sides of the bore and a hole was bored through to permit passage of the root. One or more micropotometers were cemented with DeKhotinsky to a glass rod (E) which was supported by a three-way mechanical manipulator which provided delicate adjustments and accurate placing of the micropotometers around the root. The potometers varied in length from 25 to 40 millimeters but were the same for any one experiment. Glass tubes (I and O), which pass through rubber stoppers fitted into perforations of the base, provide for inlet and outlet of glass. Moisture and airtight seals at the perforations through which the rods (D and E) passed were made with finger stalls designated as M in figure 1. These were filled with water. By packing plasticene around the base of the chamber where it fits into the groove, the interior could be completely sealed off from the exterior.

<sup>1</sup> The experiments reported in this paper were carried out in the fall of 1934 on onion sets grown the preceding spring. Observations on onion sets procured this spring (1935) show slightly higher rates of water absorption.

Throughout all of the experiments reported in this paper, air saturated with moisture was continuously passed through the chamber. Both inlet and outlet tubes (I and O) were connected to wash bottles. The latter connection served as a test to determine any leakage of air through the apparatus and as a means of gauging the relative rate of gas flow by counting the bubbles per unit time.

Most of the experiments were made on a single intact root after the sister roots had been cut off. Removing the onion from the vessel in which it grew, cutting the sister roots, and placing it in the experimental chamber, required such a short period of exposure that the root had no time to dry out in any region. This was determined by microscopic examination in a

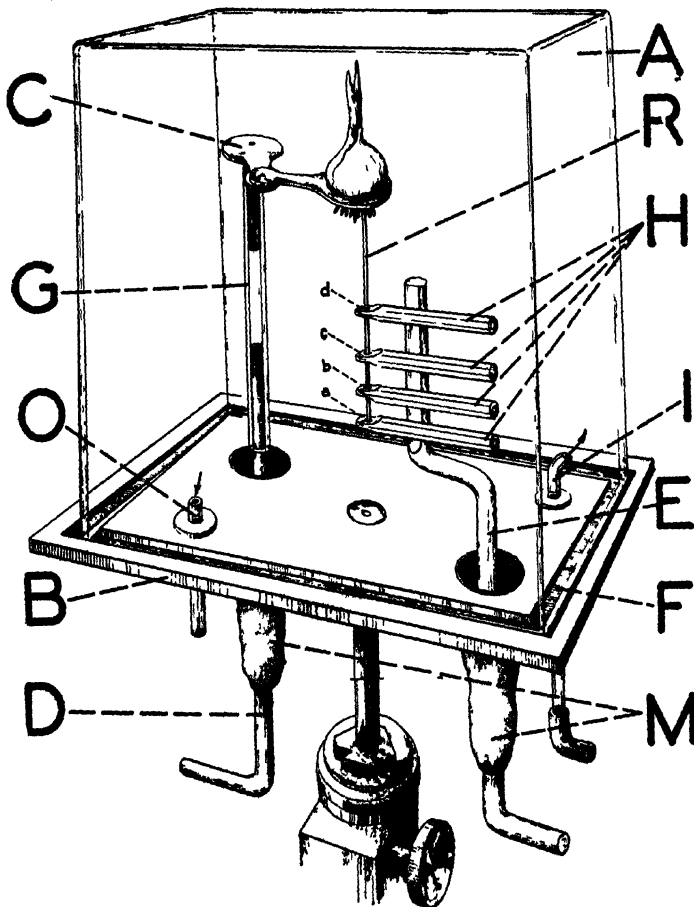


FIG. 1. Experimental chamber (description in text).

series of experiments to determine whether or not drying appeared. The root (R, fig. 1), was threaded through the openings (a, b, c, d) of the micropotometers by adjustments made with the micromanipulators on the outside, lowering the brass rod (D) and passing the root down through the openings; or by raising the glass rod (E) and passing the micropotometer tubes up over the root to the desired positions. All adjustments of the mechanical micromanipulators and all measurements were made with the aid of a horizontal microscope which was fitted with a micrometer ocular. By this means very delicate adjustments of the positions of the micropotometers could be made without mechanical injury. By raising the bulb support (D), or lowering the micropotometer support (E), at frequent intervals throughout an experiment it was possible to maintain the positions of the micropotometers at a given distance from the root tip as elongation took place. The quantity of water absorbed was determined by making consecutive minute measurements of the movement of the terminal meniscus in each tube. The height of the water column at each region was determined by measuring the distance between the upper and lower menisci around the root where it passed through the micropotometer. There was no flow of water down the root and after a period of an hour or more, depending upon the height of the water column at the beginning, the distance between the lower and upper menisci at each micropotometer contact reached a steady value which was maintained relatively constant. At the apex, where increase in diameter took place, there was a slight shift in the height of the water column. Determinations of the average diameter and height of the water column at each contact were made at frequent intervals, depending upon the nature of the experiment. No measurements were made during the first 5 to 10 hours after the onion had been placed in the chamber, in order to permit it to come into flux equilibrium with the conditions of the environment. Favorable conditions for growth were present in the experimental chamber, for the roots and leaves of the onions grew in a normal manner. New roots appeared and developed and these were sometimes used for observation. No root hairs were observed on any of the roots, whether grown in moist air, or in tap water.

Measurements were made in millimeters to the second decimal place. Although great precautions were taken when readings were made, and although the menisci at each contact appeared clear-cut and remained fairly steady after flux equilibrium conditions were reached, it might be that the absolute height of the water column was not observed. Variations of the diameters at the upper and lower menisci at a single contact did not exceed 0.02 millimeter. In all of the experiments during which the micropotometers were kept at relatively constant positions with respect to the root tip by lowering them at frequent intervals, the absolute distance from the apex



varied from one reading to the next. When adjustments were made at intervals of fifteen minutes the variation did not exceed 0.11 mm., except in a very rapidly growing root, where it reached 0.2 mm. When the interval of adjustment was longer, the variation was, of course, greater.

## Investigation

### A. ABSORPTION OF WATER BY APICAL REGIONS

#### 1. RATES OF ABSORPTION IN RELATIVELY YOUNG ROOTS 25 TO 35 MM. IN LENGTH

The quantity of tap water absorbed from 6:30 A.M. one day to 2:30 A.M. the day following, by four apical regions of the root exhibiting considerable diversity of morphological character, is shown in figure 2. The bulb with

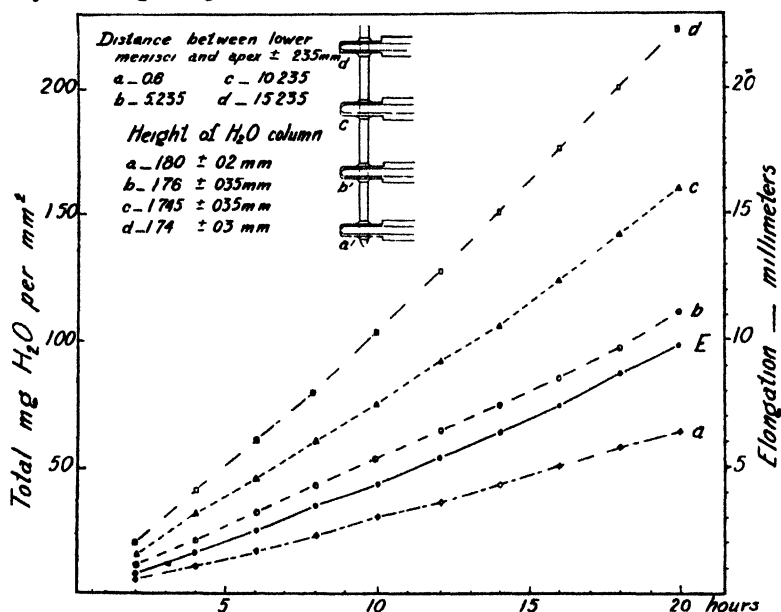


FIG. 2. Water absorbed by 4 apical regions of a single root with initial length of 25 mm. Curves a, b, c, and d show mg. of water absorbed per  $mm^2$  of absorbing surface at the micropotometer contacts a', b', c', d' shown in the root diagram. Initial diameters at a', b', c' and d' are 0.710, 0.735, 0.740, and 0.752 mm. respectively. (Curve E shows elongation in mm.

three leaves 4 to 6 cm. in length and a single remaining root had been placed in the chamber with the micropotometers in position the night before the determinations were made. At 6:30 A.M., by means of the mechanical micromanipulators on the outside, the micropotometers were lowered until the position of each was respectively 0.4, 5.0, 10.0, and 15.0 mm. from the apical point of the root. Every half hour or less, the micropotometers were lowered with respect to the growing root in order to maintain them in the

same relative positions. Measurements of the root diameters at each meniscus of the water columns, the height of the water column at each region, the movement of the terminal meniscus in the micropotometer, and elongation were recorded every four hours. The height of each water column with the maximum variations between readings, the distance between the lower meniscus at each micropotometer and the apex, and the average diameter of each contact are given in figure 2. Elongation is shown by curve *E*. The rate of elongation is fairly uniform, tending to increase during the night, especially after midnight. Curves *a*, *b*, *c*, and *d* (fig. 2), show the milligrams of water absorbed, calculated in terms of unit surface exposed to the water of each micropotometer.

Curve *a* represents the quantity of water absorbed by the surface in a region where active cell division is taking place, and tissue differentiation is at a minimum; curve *b* shows the milligrams of water absorbed by the surface in the fifth and sixth millimeters which is the zone just proximal to the region of elongation, and curves *c* and *d* give the quantity of water absorbed by regions relatively older where tissue differentiation has taken place and vacuolization tends to reach a maximum.

The curves clearly show that water was absorbed by the surface in all four of the regions mentioned above, and that the rates of absorption in the regions were very different. Greater quantities of water were absorbed by the relatively more basal regions. A comparison of the slopes of all of the curves shows that there was an increase in the rate of absorption in each region with time, the most rapid rates appearing toward the end of the experiment. In this experiment, which is typical, the greatest increase in the velocity of absorption of any one zone occurred in the region which is in the 15th and 16th millimeter from the apical point of the root.

When all of the roots are left intact, the quantity of water absorbed by any region of one of the roots is less than if all of the roots but one are cut off. This fact is shown by the curves in figure 3, which give typical results obtained when measurements were made on a given root with (1) all of the sister roots intact and (2) all of the sister roots removed. In this case, the observations were made on a bulb which had three leaves 3 to 5 cm. in length, and fourteen roots 10 to 30 mm. long. Three micropotometers were placed on a root which was 27.3 mm. in length in the positions designated as *x'*, *y'*, *z'* in diagram N (fig. 3). These positions corresponded to the regions (1) where active cell division was taking place; (2) where elongation was at a maximum; and (3) the zone proximal to the region of elongation. No observations were made until 8 hours after the bulb had been placed in the chamber. From then on, measurements were made every four hours, and adjustment for elongation was made at half-hour intervals except during the night. The two morning readings, therefore, showed

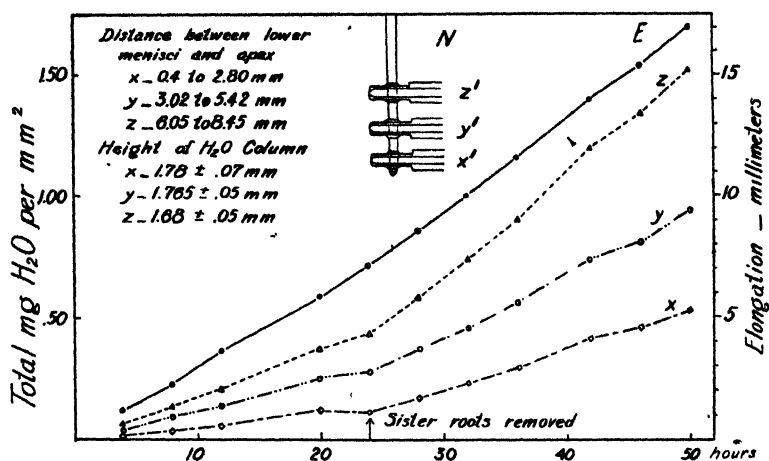


Fig. 3. Absorption of water by 3 apical regions of a single root when (a) all the roots were intact and (b) the sister roots were removed. Curves  $x$ ,  $y$  and  $z$  show the mg. of water absorbed at the contacts of the micropotometers  $x'$ ,  $y'$  and  $z'$  illustrated in diagram N. Diameters at contacts of the micropotometers  $x'$ ,  $y'$ ,  $z'$  were 0.77, 0.787 and 0.80 mm. respectively. Curve  $E$  shows elongation. The arrow on the base line indicates time at which the sister roots were removed.

higher values since these represented the absorption of water during an interval when the positions of the micropotometers relative to the base varied from 2 to 2.5 mm. After 24 hours, as designated by the arrow on the base line in figure 3, all of the roots but the one which was in contact with the water in the micropotometers, were quickly cut off with sharp scissors without changing the positions or the terminal menisci of the micropotometers in any way. The heights of the water columns are tabulated in figure 3. Maximum variation during any one interval was 0.07 mm. which occurred at the apical micropotometer the second night. The limits of variation in the positions of the micropotometers relative to the apex of the root, and the average diameter at each contact, are also tabulated in figure 3. As shown by curves  $x$ ,  $y$ , and  $z$  (fig. 3) tap water was absorbed by the surfaces in all three of the regions mentioned above. Pronounced change in the slopes of the latter half of each curve demonstrates that after the sister roots had been removed, greater quantities of water were absorbed by all of the surfaces exposed to tap water in the micropotometers. Ten other experiments similar to the above, with the micropotometers in various positions, showed essentially the same results. In each case, the rate of absorption in the single intact root was greater after removal of the sister roots, and the greatest quantity of water was absorbed by the region nearest the base. In all of the experiments in which measurements of the absorption of water were made on a single intact root with the sister roots removed,

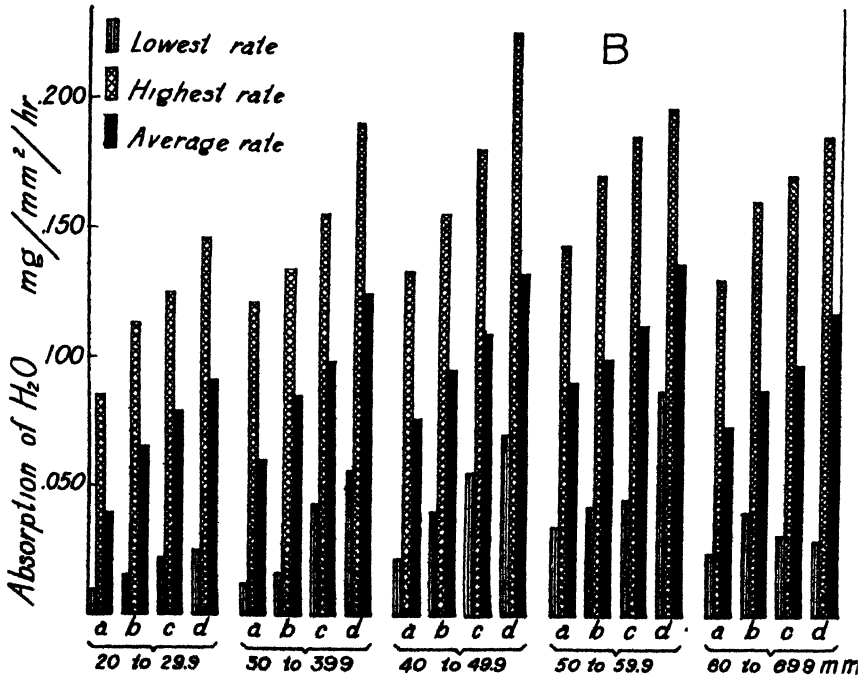
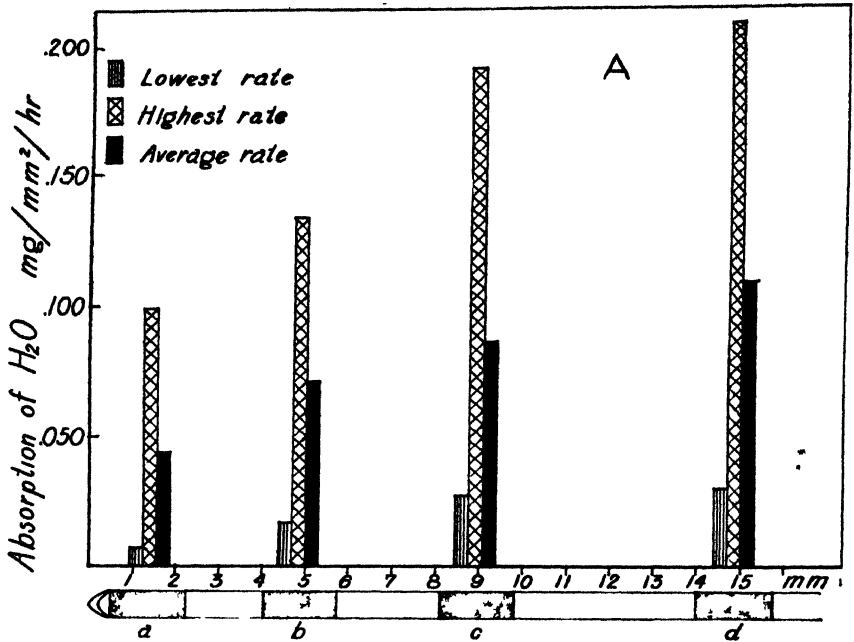
the observed rates of absorption were relatively higher than when the sister roots were present, but the distribution of the velocities of absorption remained the same. Changes in the distribution of the rates of absorption occurred but these were related to other phenomena, not to the presence or absence of sister roots.

By using a greater number of micropotometers, by placing them close together, and by changing the positions in different experiments, it was found that all regions of the root between the root cap and 16 mm. from the apex absorbed water. The highest rates observed were exhibited by the region 14 to 16 mm. from the apex. All of the roots exhibited a distinct unidirectional gradient of increase in the rates of water absorption by unit surface areas from the apex towards the base. However, individual roots show considerable diversity in water-absorbing power. The results of measurements made on 10 roots with initial lengths of 25 to 32 millimeters, continuing throughout a fourteen-hour observation period, and using four micropotometers are given in figure 4, A.

Frequent adjustments (15 to 30 minute intervals) of the micropotometers were made in order to maintain them in the positions designated by the stippled areas (*a, b, c, d*) in the diagram of the root illustrated in figure 4. The greatest variation in any one experiment from the regions designated was 0.5 mm. in a rapidly growing root. Measurements were made every two hours, and calculations were made of the average rate of absorption in milligrams of water per hour per square millimeter of surface exposed to the water in the micropotometers. The highest and lowest rates given in graphic form in figure 4 A, are the rates of absorption in four regions of two different roots and are the result of averaging seven determinations throughout the fourteen-hour period. On the other hand, the graphic representation of the average rates in figure 4 A, are the average rates of all measurements made on the designated regions in 10 different roots.

Several interesting facts are apparent when a comparison is made of the heights of the bars in figure 4 A. In the first place, the comparison shows that there is a wide variation between the highest and lowest rates observed in any one region; in the second place, it shows that in spite of the large difference between these two roots which exhibit the highest and lowest rates, each individual root manifests a distinct functional polarity with respect to the distribution of the rates of absorption in these regions; and in the third place, the polar distribution of the rates of water absorption is also shown by comparing the average rates of all of the regions in all of the roots.

A comparison of the percentage differences in the average rates of absorption of the four regions in all of the roots shows that the region between 14 and 16 millimeters from the apex absorbed water at a rate which



was 26.4 per cent. greater than the region between 8 and 10 millimeters, 54.9 per cent. greater than the region between 4 and 6 millimeters, and 150 per cent. greater than the region 0.5 to 2.5 millimeters from the apex. The region between 8 and 10 millimeters absorbed water at a rate which was 22.5 per cent. greater than the rate in the region between 4 and 6 millimeters, and 17.7 per cent. greater than the region at the extreme apex; and the zone between 4 and 5 millimeters absorbed water at a rate 61.3 per cent. greater than the most apical region designated as *a* in the diagram of the root in figure 4 A. These four regions were selected for special study because they are the regions which determine the characteristic distribution of bioelectric potential in the onion root.

## 2. EFFECT OF AGE ON THE RATES OF ABSORPTION IN APICAL REGIONS

In order to determine the effect of age, measurements of the rate of absorption were made throughout a growth period when the roots elongated from an initial length of 20 mm. to 90 mm. or more. The micropotometers were maintained in the relative positions designated as *a*, *b*, *c*, and *d* in the diagram of the root (fig. 4 A) by moving them at intervals of an hour during the day and 6 hours at night. The values obtained at the first morning determinations were therefore a little high. The greatest shift in the position of the micropotometers in a single root was 4 millimeters, the average for all roots, 2.4 millimeters. The micropotometers had to be refilled during the course of the experiments but this was done very quickly with a pipette and no apparent drying of the root occurred.<sup>2</sup> Measurements were made every 4 to 6 hours. In order to compare the change in the rate of absorption of each region with age, the average rates of each region in each root during a growth interval in which the root increased in length from 9 to 9.9 mm. (to be designated as a 10-mm. growth period) were determined. Measure-

<sup>2</sup> Recent modifications of the apparatus permit refilling of the micropotometers, and removing any collected fog on the glass wall facing the microscope, without changing the humidity of the interior of the chamber.

FIG. 4. Comparative rates of absorption in different roots. A. Rates of water absorbed by 4 apical regions of 10 different roots during a 14-hour observation period. Initial lengths of roots were 25 to 32 mm. The positions of the micropotometers are indicated by the stippled areas *a*, *b*, *c*, and *d*, in the diagram of the root while the vertical bars above represent rates of absorption in the corresponding region. Bars with vertical lines give rates of absorption in the root which manifested the lowest rate, bars with crossed lines give rates of absorption in the root which manifested the highest rate (each bar is average of 7 observations). Solid black bars give average rates in the designated regions during the 14-hour period for all roots. B. Change in rate of water absorption with age. Vertical bars represent the rates of absorption during 5 growth intervals by each region corresponding to *a*, *b*, *c*, and *d* in the diagram of the root. Bars with vertical lines and crossed lines give lowest and highest rates respectively, solid black bars give average rates of 11 different roots. Growth intervals are designated in mm. below the vertical bars.

ments were made on many roots. The exact time at which the 9 to 9.9 mm. increase in length was reached was not observed in all cases, since on certain nights the time interval from one reading to the next was 6 hours. Only the results obtained from eleven roots, in which the transitional changes from one growth interval to the next were observed, are included in the graphic representation in figure 4 B. Tables of detailed measurements are omitted in order to save space.

The highest and lowest average rates observed in individual roots during the 10-millimeter growth period are represented graphically in figure 4 B by the vertical bars with the crossed lines, and with the vertical lines, respectively. A comparison of the heights of the bars with vertical lines shows that in the corresponding root the increase in rate of absorption of each region for each 10-mm. growth period was not uniform. Regions *a* and *b* in this instance reached a maximum rate during the 50 to 59.9-mm. growth period, region *c* in the 40 to 49.9-mm. growth interval, and region *d* in the 50 to 59.9-mm. interval. A comparison of the heights of the crossed bars show that in another individual root region *d*, which was 14 to 16 mm. from the apex, reached a maximum earliest (40 to 49.9-mm. interval), and regions *c*, *b*, and *a* followed in the next interval (50 to 59.9-mm.). The results obtained from measurements on these two roots demonstrate that individual variations exist and that a given region does not necessarily exhibit a maximum rate of absorption when another region (or regions) of the same root reaches a maximum rate. However, characteristic changes are the rule. The majority of roots tend to manifest maximum absorption rates when they reach lengths between 40 and 60 mm. as shown by a comparison of the heights of the solid black bars in figure 3 B. These represent the average rates of absorption in the designated regions for all of the roots during five growth intervals. Since during the night there was a relatively greater increase in the distance of each water column from the apex, this would tend to make the average rates of absorption somewhat higher at each region than if the positions of the micropotometers relative to the apical point did not change. Exceptions were found, especially in relatively short roots (less than 40 mm.) which developed late and grew slowly, and in relatively long roots (over 60 mm.) which developed early and grew rapidly. Calculations of the percentage difference in the average rates of absorption in each region for all the growth periods are tabulated below.

1. Region *d* which was 14 to 16 millimeters from the apex.

Growth intervals in mm.	Percentage differences
20 to 29.9 and 30 to 39.9	36.0 per cent. increase
30 to 39.9 and 40 to 49.9	6.4 per cent. increase
40 to 49.9 and 50 to 59.9	3.0 per cent. increase
50 to 59.9 and 60 to 69.9	16.2 per cent. decrease

2. Region *c* which was 8 to 10 millimeters from the apex.

Growth intervals in mm.	Percentage differences
20 to 29.9 and 30 to 39.9	24.1 per cent. increase
30 to 39.9 and 40 to 49.9	11.2 per cent. increase
40 to 49.9 and 50 to 59.9	2.75 per cent. increase
50 to 59.9 and 60 to 69.9	15.4 per cent. decrease

3. Region *b* which was 4 to 6 millimeters from the apex.

Growth intervals in mm.	Percentage differences
20 to 29.9 and 30 to 39.9	30.7 per cent. increase
30 to 39.9 and 40 to 49.9	11.7 per cent. increase
40 to 49.9 and 50 to 59.9	4.2 per cent. increase
50 to 59.9 and 60 to 69.9	13.7 per cent. decrease

4. Region *a* which was 0.5 to 2.5 millimeters from the apex.

Growth intervals in mm.	Percentage differences
20 to 29.9 and 30 to 39.9	53.8 per cent. increase
30 to 39.9 and 40 to 49.9	25.0 per cent. increase
40 to 49.9 and 50 to 59.9	18.4 per cent. increase
50 to 59.9 and 60 to 69.9	23.2 per cent decrease

## B. ABSORPTION OF WATER BY RELATIVELY MORE BASAL REGIONS

By placing a large number of micropotometers on a given root, and by shifting the positions of the micropotometers when desired, determinations of the rates of absorption in all the root regions between the root cap and the bulb were made. It was found that all the root regions from the root cap to the base absorbed water, but at different rates, and that the distribution of the rates of absorption was not the same for all roots. The determinations demonstrated that in roots less than 50 mm. in length, there was in general an increase in the rate of absorption per unit surface from apex to base, and the highest rates occurred in the regions nearest the bulb; whereas in roots over 70 mm. in length, the regions of maximum absorption were relatively more apical. In the majority of roots over 80 mm. in length, maximum rates were observed in regions within the first 40 mm. from the apex. Although experiments have been made on many roots of different lengths and age, it was found that all of the root regions between the root cap and the base absorbed water. The longest root studied was 220 mm. in length. Most roots tend to develop lateral roots at the age of 2 to 3 weeks. It was observed that there was a definite shift of the region of maximum absorption rate towards the apex, with the development of lateral roots. It is entirely possible and highly probable that the regions of the



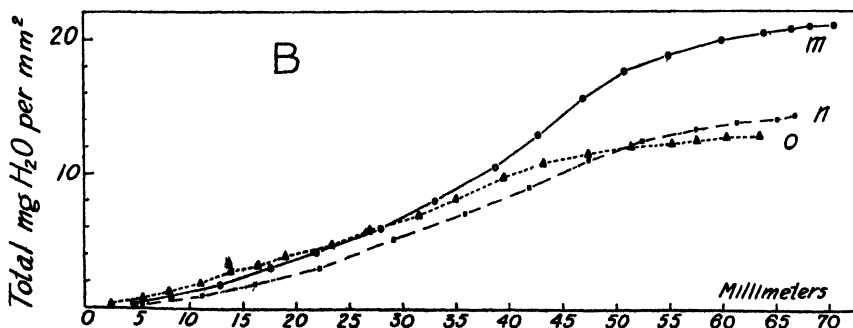
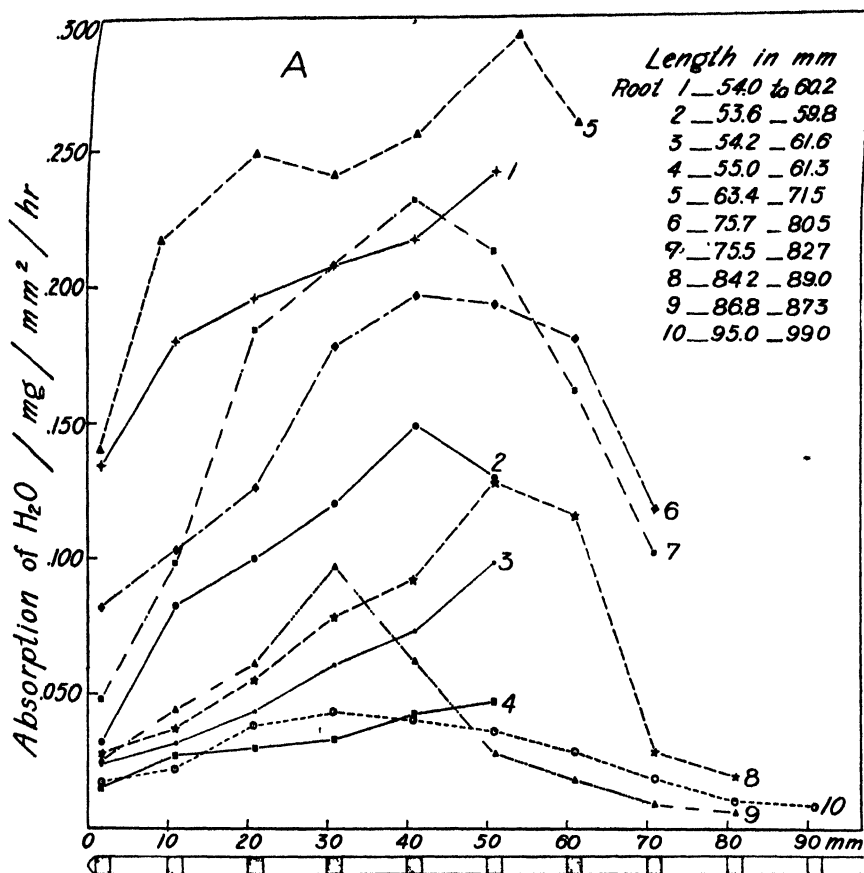


FIG. 5. A. Rates of water absorption in 10 selected regions of 10 different roots during a 12-hour observation period. Curves 1 to 10 represent the average rates of water absorption by 10 different regions in ten different roots corresponding to the numbers on

root not exposed to the water of the micropotometers also absorbed water since they were in a saturated atmosphere.

Figure 5 A shows the rates of water absorption in selected regions of ten roots, the initial lengths of which varied from 54 to 95 mm. The positions of the micropotometers were maintained relatively constant with respect to the apex of each root by moving them at intervals of one-half hour or less. Measurements were made every two hours during a 12-hour period, and the average rate of absorption for each 12-hour period was calculated and plotted on a graph as shown in figure 5 A. The initial and final lengths of each root are given in figure 5 A, after the first column of numbers, which correspond to those of the curves of distribution. The stippled areas in the diagram of the root below the curves represent the positions of the micropotometers which were placed 10 mm. apart. A comparison of all of the curves shows that the highest rates of absorption are exhibited by relatively more basal regions, and that the region where maximum rates occur varies in individual roots, but is closely linked with the lengths of the root. The initial lengths of the 4 roots which exhibited the rates given by curves 1, 2, 3, and 4 (fig. 5), were less than 60 millimeters. A comparison of the slopes of these curves demonstrates considerable individual differences in the rates of absorption of different roots, but all manifest an increase in the rate per unit surface from apex to base. Note that in curve 5 (fig. 4 A) there are variations in the distribution of the rates of water absorption, although the highest rates relative to the apex are observed in the basal regions. As shown by curves 1, 2, 3 and 4, maximum rates of absorption are found at or very near the base in roots of this length. These roots were 10 to 15 days old. Curves 5, 6, 7 (fig. 5 A), which represent measurements on roots 63 to 75 mm. in length, show that comparatively rapid rates of absorption are exhibited in all regions and that the maximum rate appears in regions nearer the apex. The experiments show that, with increasing age, the regions of maximum absorption shift toward the apex. This fact is further emphasized by comparing curves 1 to 8 with curves 8, 9, and 10, obtained from experiments on roots over 80 mm. long.

The above results and those depicted in figure 4 B indicate that each root region exhibits an increase in the rate of absorption, which goes through a maximum with age. Experiments to prove this were made, and the results are given by curves *m*, *n*, and *o* (fig. 5 B). Each curve represents the

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the right of the figure. The corresponding initial (first column) and final lengths (second column) of each root are given after the root numbers. Stippled areas of the root in the diagram below the curves show the positions of the micropotometers. B. Change in quantity of  $H_2O$  absorbed by a given region with age. Curves *m*, *n*, and *o* obtained from 3 different roots show the mg. of water absorbed by a single region during an observation period when the distance between the fixed micropotometer and the apex increased with growth.

rates of absorption in one region of a single root. The initial lengths of the roots which correspond to curves *m*, *n*, and *o*, were respectively 20.3, 27.0, and 32.6 millimeters. In each experiment, after the onion bulb had been in the chamber 8 hours, the position of the single micropotometer used was adjusted so that the lower meniscus was within 0.5 mm. of the root tip. Measurements were made at 10-hour intervals. Since the position of the micropotometer on each root was not changed, the distance between it and the growing root tip increased with increase in root length as the root grew, but remained the same distance relative to the base of the root, after fifteen hours or less, depending upon the rate of elongation. The slopes of the curves *m*, *n*, and *o*, all show a typical sigmoid curve. It is obvious that at each region there was an increase in the rate of absorption with age, and that each region passed through a maximum when it was from 40 to 60 mm. from the apex. These experiments corroborate the conclusions given above that the distribution of the velocities of absorption depends upon the age of the root.

### Discussion

The experimental evidence which has been presented heretofore regarding the absorption of water by the apical meristem is not in agreement. PRIESTLEY and TUPPER-CAREY (5) showed that the apex was relatively impermeable, HÖHN (1) that comparatively little absorption occurred, while SIERP and BREWIG (8) maintained that the first 5 mm. of the apex not only showed an absence of absorption, but that it actually gave off water. A large number of investigators, including POPESCO (4), URSPRUNG and BLUM (9), KELLER (2), and HÖHN (1), claim that the chief absorbing zone is the region just proximal to the zone of elongation. KELLER (2) maintains that the distribution of the rates of water absorption in the root corresponds to the distribution of bioelectric potentials. Most of the work on absorption rates has been carried out on roots other than those of the onion. Just as individual roots exhibit variations in the velocities of absorption and their distribution, it is to be expected that differences will be found in the roots of different plant species. However, such differences would not account for the pronounced lack of agreement found among the investigators mentioned above.

It is doubtful if much value can be placed on the recent experiments carried out by SIERP and BREWIG (8) since the roots were stimulated by inverting the normal orientation with respect to gravity and by wrapping the roots at intervals with cotton thread. It does not seem likely that the apical meristem gave off (excreted?) water unless disintegration of the tip occurred. The authors state (8, p. 107) that the increase in the micropotometer volume was at least 149 mm.<sup>3</sup> whereas the increase in the volume of the root apex resulted in a maximum of 3 mm.<sup>3</sup> but they do not state how

they determined the increase in root volume. Measurements have been made by the author on the roots of onions maintained in the inverted position but no such unique phenomenon has been observed. On the contrary, the regions between the root cap and base all absorbed water and the polar distribution of the rates of absorption was maintained. However, in these experiments, transpiration was at a minimum. SIERP and BREWIG on the other hand, enclosed the inverted stems of their plants in a chamber and varied the humidity.

SIERP and BREWIG also state that, with increase in transpiration rates, there is a migration of the region of maximum absorption toward the apex. They do not, however, show whether this shift is dependent on age, as it is in the onion root. Owing to the fact that the onion bulb provides a reservoir of water, the onion does not furnish good material to determine the relations between water absorption and transpiration. Nevertheless the writer found that in the onion root there was an increase in absorption at all of the root regions under certain conditions when transpiration was increased; but the polar distribution of the rates of water absorption was always maintained.

HÖHN (1) claims that the entire root surface of the roots he studied (*Zea mays*, *Triticum vulgare*, *Tradescantia fluminensis*) absorbed water. As mentioned above, he failed to make simultaneous measurements of water absorption in a single root, and his tabulated data do not support the conclusions he has made. HÖHN maintains that the hourly water absorption of an old root, calculated on the basis of unit length, is greater than that of a younger root in most of his experiments; but his tables do not warrant such an interpretation. Table 8 (1, p. 550) shows, for example, that a root 65 mm. in length absorbed 0.43 mg. of water per hour per mm. length of the root, whereas a root less than half this length (26 mm.) absorbed 0.89 mg. of water per hour per unit root length. It may be that the shorter root had grown slowly and was actually the older root, but HÖHN presents no evidence in favor of this suggestion. Table 9 (1, p. 551) shows that a root 58 mm. in length absorbed 0.62 mg. of water per unit length in unit time, while another root 25 mm. in length absorbed 1.16 mg. of water. The longer root, which was over twice the length of the shorter, absorbed water at half the rate. HÖHN's tables merely establish the fact that individual differences exist with respect to the rates at which different roots absorb water. Although HÖHN states that he used "similar" roots, his data furnish no evidence in support of his statements, since no measurements of root diameter were made.

KELLER (2) used dyes to determine the rates of water absorption in the root, and reached conclusions similar to those of POPESCO (4). In favor of his hypothesis that electro-osmotic forces are involved in water absorption and transport, KELLER calls attention to the correspondence of their

results—which maintain that the main absorbing region of the root is the region of elongation—with those of LUND (3) on bioelectric potentials in the onion root, which show that the region of elongation is electronegative to other regions of the root.

The results presented in this paper of direct quantitative determination of the rates of water absorption in the onion root itself show that there is no correspondence between the unidirectional gradient of distribution of the velocities of water absorption in roots less than 50 millimeters in length, and the distribution of electric potentials in roots of similar length as shown by LUND and co-workers (3, 7). The region of relatively low electronegativity from 4.5 mm. to between 7.5 to 14 mm. is not the region which exhibits either a maximum or minimum rate of absorption except in very old roots (over 3 weeks) and then rarely. The fact that the gradient of the distribution of electric potentials in the onion root does not correspond to the gradient of the distribution of velocities of water absorption does not necessarily indicate that electric energy is not utilized in the processes of absorption and transport. Experiments to determine possible linkage between the production of electric energy and the absorption of water in the onion root are being carried out at the present time.

The most striking facts brought out in this paper are that in the onion root, all root regions between the root cap and the base absorb water at different rates, that the rates of absorption per unit area change with time, going through a maximum. The precise measurements show definitely that the distance from the apex to the exact region which exhibits the highest rate of absorption differs in individual roots and changes with age.

The present paper makes no attempt to consider the mechanism or mechanisms involved in the absorption of water; but it furnishes for the first time precise quantitative data concerning the distribution of the velocities of water absorption in a single intact root under carefully controlled conditions.

### Summary

1. A technique is described by means of which precise quantitative data on the velocities of absorption of tap water by different root regions of the same intact root may be simultaneously determined under carefully controlled conditions.

2. All root regions of the onion root between the root cap (on which no measurements were made) and the bulb absorb tap water but at unequal rates.

3. In relatively young roots (less than 50 mm.) there is a unidirectional gradient of the distribution of velocities of water absorption, the region of maximum absorption appearing at the base.

4. In relatively older roots (more than 70 mm.) there exist two pronounced unidirectional gradients with maximum absorption velocities appearing in regions 40 to 60 mm. from the apex.

5. The velocities of absorption of water are less in all root regions between the root cap and base in any one root when all of the sister roots are present. Removal of sister roots increases the rates of absorption in all root regions of the remaining root.

6. Each root region goes through a maximum with respect to age, exhibiting highest velocity rates when the root is from 40 to 60 mm. in length.

7. With increase in age there is a shift of the region exhibiting maximum rates toward the apex.

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# RELATION OF NUTRIENT SALT CONCENTRATION TO GROWTH OF THE TOMATO AND TO THE INCIDENCE OF BLOSSOM- END ROT OF THE FRUIT<sup>1</sup>

W. REI ROBBINS

(WITH EIGHT FIGURES)

## Introduction

The control of the nutrient salt concentration of the solution bathing plant roots is a factor of importance in plant growth whether in artificial culture or in soil. When essential salts are present in very low concentrations, growth may be limited by a deficiency of one or more of the elements necessary in plant metabolism. When salt concentrations are high, growth is dependent upon factors other than mere quantitative nutrient supply. One of these factors is the osmotic concentration of the solution which, in turn, affects the water relations within the plant.

In the tomato plant, a serious disturbance of the normally existing water relations has been assigned as the cause of the physiological disease of the fruit known as blossom-end rot. This disease consists of the development at the stylar end of the fruit of a spot having a slight, watery discoloration of the tissue which increases in size and gradually turns from brown to black. It is accompanied by a shrinkage of the affected part of the fruit. The symptoms of this disease are known, its occurrence has been frequently described, and the serious loss which it causes in some seasons is well recognized (1, 3, 15, 28, 29, 35).

Although the development of blossom-end rot is evidently concerned with the adequacy of the water supply of the affected tissues of the fruit, yet many other factors than the concentration of the nutrient solution have been indirectly associated with the incidence of this fruit disorder in the garden, field, and greenhouse. Low soil moisture has been the factor most often associated with the development of blossom-end rot of the tomato (3, 29, 35). Various other cultural or environmental factors have likewise been associated with the incidence of this disease as: over-watering the soil (15), and the attendant lack of gaseous exchange in the soil following heavy watering (28); the staking of tomato plants (29, 30); the use of unbalanced fertilizers high in potassium and ammonium salts (1, 35); and the transfer of tomato plants from a shaded to a non-shaded greenhouse (3). Vigorously vegetative plants have been found to be more generally affected with blossom-end rot than plants grown less vigorously (3, 15). On the other hand,

<sup>1</sup> Journal Series paper of the New Jersey Agricultural Experiment Station, Department of Plant Physiology.



shading the plants (35), and the use of nitrate rather than ammonium nitrogen salts as constituents of the fertilizer have been thought to reduce the incidence of blossom-end rot (3, 29, 35).

The idea has been suggested (35), that this malady is related to transpiration and that there seems to be competition between the fruits and the leaves for water, and that when wilting of the leaves begins, water may then be drawn from the fruits. In fact, WILSON and RUNNELS (36, 37) have shown that the well known increase in the rate of transpiration accompanying the application of a Bordeaux mixture spray was associated with an increased percentage of fruits showing blossom-end rot compared with that of unsprayed plants. On the other hand, they found that a heavy oil spray reduced both the transpiration and the percentage of fruits showing blossom-end rot as compared with that on unsprayed plants. However, there seems to be no general appreciation of the relation between the concentration of the solution surrounding the roots and the incidence of blossom-end rot of the tomato fruits.

The object of this paper, therefore, is to study the relation of the salt concentration of the nutrient solution to the growth of the tomato plant and to the incidence of blossom-end rot of the fruit, and to show how various other factors of nutrition and environment may be important in modifying these relations.

### Materials and methods

The general plan of the experiment consisted of growing tomato plants in sand culture with complete nutrient solutions of different total salt concentrations; recording the resulting growth responses and making such determinations which seemed significant with respect to the relation between the concentration of the nutrient solution and the growth of the plant and to the development of blossom-end rot of the fruit.

Seeds of the progeny of a single Marglobe tomato plant were germinated in sand beginning July 3, 1934. The Marglobe variety was used, not only because of its great commercial importance but also because this variety is known to be relatively less susceptible to the development of blossom-end rot than many other varieties, particularly Stone, Dwarf Stone, and Baltimore (29, 35). It was thought, therefore, that any effect of the concentration of the solution upon the incidence of blossom-end rot in this variety would probably be more marked with many other varieties. Forty-eight carefully selected seedlings were transplanted on July 24 into thoroughly washed quartz sand in 2-gallon glazed crocks commercially known as coffee urn linings, one plant per crock. Two somewhat different grades of sand were used for comparison. One grade of sand was of medium fineness, from which all particles larger than 14-mesh and most all particles smaller than

TABLE I

MECHANICAL ANALYSIS OF TWO GRADES OF SAND USED, GIVING PERCENTAGE OF DIFFERENT SIZED PARTICLES

PARTICLE SIZE	SAND NO. 1	SAND NO. 2
	%	%
Larger than 2 mm.	0.0	2.3
2 mm. to 1 mm.	35.0	41.7
1 mm. to 0.5 mm.	63.7	53.3
Smaller than 0.5 mm.	1.3	2.7

40-mesh to the inch had been removed by screening; the other was a slightly coarser sand used without screening. The distribution of the various sized particles in each grade used is given in table I. All plants were initially supplied with the same complete nutrient solution of approximately one-quarter atmosphere osmotic value until August 14, when the plants had become well established. They then averaged 20 cm. in height, with a stem diameter of 5-6 mm., and had an average of 5 to 6 leaves each. At this time, six different nutrient treatments comprising six series with eight plants in each series were initiated, using complete nutrient solutions of five different salt concentrations. The cultures were replicated and so situated in the greenhouse as to eliminate, as far as possible between series, the effects of differences in the environmental factors of light, humidity, temperature, and air movement on the growth of the plants. These series will be designated A to F inclusive. All cultures in series A and B received the same solution of lowest concentration, namely, 0.08 atmosphere osmotic value, but at different rates of supply. Cultures in series C to F inclusive, received solutions of 0.44, 0.83, 1.7, and 3.1 atmospheres osmotic value, respectively. The rate of nutrient solution supply was approximately one liter per plant per day in all series except series A, which was four liters daily. This higher rate was employed to reduce to a minimum any nutrient deficiency which with the solution of this low salt concentration might limit growth if supplied at the lower rate as in series B. Table II gives the partial volume molecular concentrations and the parts per million of the salts, together with the osmotic and pII values of the solutions used in each series. The nutrient solutions were applied to the sand in each culture continuously by the method of SHIVE and STAHL (23). The relative proportion of the salts, which was the same in all the solutions except the lowest in concentration, had previously been found satisfactory for the growth of the tomato plant (4). Both nitrate and ammonium nitrogen were used in these solutions in order to reduce to a minimum the change in pH value of the solution which takes place during growth due to differential ion absorption.

TABLE II  
NUTRIENT SOLUTIONS USED IN SERIES A TO F

NUTRIENT SOLUTION		NUTRIENT SALTS				
SERIES	OSMOTIC VALUE	TOTAL	KH <sub>2</sub> PO <sub>4</sub>	Ca(NO <sub>3</sub> ) <sub>2</sub>	MgSO <sub>4</sub>	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>
	<i>atm.</i>	<i>p.p.m.</i>	<i>Partial volume molecular concentrations</i>			
A & B	0.08	190	0.00026	0.00037	0.00050	0.00018
C	0.44	997	0.00106	0.00146	0.00356	0.00140
D	0.83	1,992	0.00211	0.00292	0.00711	0.00280
E	1.70	4,978	0.00528	0.00730	0.01775	0.00700
F	3.10	10,055	0.01055	0.01460	0.03550	0.01400

Each culture of a series was flushed daily with the solution used in that particular series to prevent salt accumulation. Daily records were taken of the maximum and minimum temperatures, the wet and dry bulb temperatures, and the evaporation from both black and white standardized spherical atmometer cups (13). The percentages of relative humidity were obtained from the wet and dry bulb thermometer readings with the aid of published tables (17). To aid good pollination, plants were jarred at appropriate times. On September 21, one-half of the plants in each series was harvested. Nutrient treatments were continued with those cultures remaining. The pH values of the nutrient solutions were obtained colorimetrically.

The osmotic values of the nutrient solutions and of the extracted plant juices were determined by means of the freezing point depressions of the liquids, with the Beckman thermometer according to the method of HARRIS and GORTNER (9). Appropriate corrections for undercooling were made. All reported osmotic values are those at 20° C. The tissue fluids used for the determination of osmotic values were obtained by extraction with a hand screw press from the representative tissue samples.

Determinations of refractive indices were made at controlled temperature with a Zeiss Abbe refractometer following the suggestions of GORTNER and HOFFMAN (7) with regard to plant tissue extracts. The values of water content of the tissues were obtained from SCHÖNROCK's table (21). The plants were harvested on the dates indicated and the tissues were rapidly dried in a current of air at about 70° C. The analytical fraction reported "leaves" included the petioles. The fraction reported "stems" included only that portion of the stem produced as new growth after the nutrient treatment in each of the several series was started. For the determination of osmotic values of tissue extracts, the fraction reported "stems" included both young stem and leaf tissues and the fraction designated "fruits" consisted of green, immature fruits from 2.5 to 3.5 cm. in diameter.

## Experimental results

### ENVIRONMENTAL CONDITIONS

Daily records made of certain factors of the environment during the course of the experiment are given in figure 1. The lower curves in the diagram represent corrected values for the loss of water, in grams, by evaporation from the surface of the black and white Livingston standard spherical atmometer cups for each twenty-four-hour period. These values indicate wide differences in daily evaporating power of the air. For example, the daily rate of evaporation on September 12 was 36 gm. for the black cup, and 29 gm. for the white cup. Five days later, on September 17, the value for each cup was less than 4 gm. The days with very low evaporation rates were usually either very cloudy or rainy, accompanied by a somewhat lower temperature and high relative humidity. The days with high evaporation rates were usually those of high light intensity, accompanied by high temperatures and low relative humidity. Although no direct quantitative measure of light intensity itself was made, the differences between

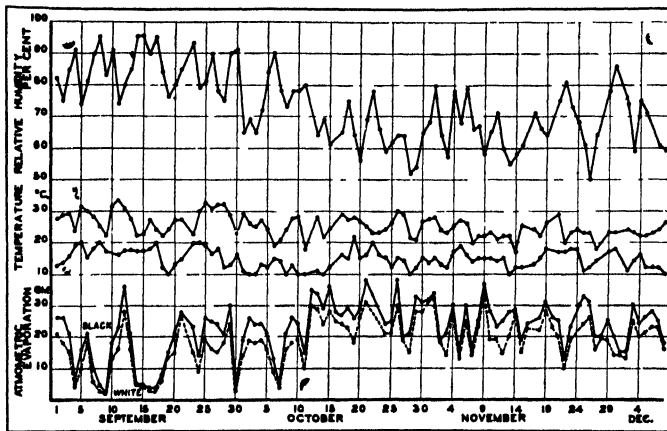


FIG. 1. Daily records of percentage relative humidity, maximum and minimum temperature, and rates of atmospheric evaporation from standard black and white atmometers.

the daily evaporation from the black and white atmometer cups reflect the relative amount of absorption of solar radiant energy, and vary directly with changes in light intensity (12). On bright days these differences were relatively large, for example, on September 12, September 25 to 29, October 1 to 4, October 8 to 10, and October 21 and 26, as indicated in figure 1. On dark days these differences were relatively small; for example, on September 8, 9, 14, 17, and 30, and October 7. Since the absorption of radiant energy greatly increases transpiration, as well as evaporation, the effect of light on transpiration has an important bearing upon the relation between the con-

centration of the solution and the occurrence of blossom-end rot of the fruit. Throughout the experiment there continued to be fluctuations from day to day in evaporation rates, but with the fall in the dewpoint and decrease in actual humidity of the outside air with the coming of cool weather, and with the artificial heating of the greenhouse in which this experiment was conducted, the average relative humidity in the greenhouse gradually decreased during October and November. Consequently, the average evaporation rates were higher than during September and early October.

The percentages of relative humidity were obtained from wet and dry bulb thermometer readings taken at 8:30 A. M. each day, and are represented on the upper curve in figure 1. Extreme air currents were not present in the greenhouse, and, therefore, the evaporation rates varied inversely with relative humidity in most cases. The values for percentage relative humidity, shown in figure 1, however, give a measure of the vapor pressure deficit at the time of the reading, whereas the values of evaporation rates given, are twenty-four hour rates. Therefore, while sudden changes in temperatures and absolute humidity taking place during the day are not reflected in the values for percentage relative humidity given in figure 1, nevertheless the results of such changes are integrated in the values for the twenty-four-hour rates of evaporation. The curve indicating relative humidity shows the wide fluctuation in values within periods of a few days duration; for example, from 91 per cent. on September 30 to 65 per cent. on October 1, and again from 78 per cent. on October 22 to 25 per cent. on October 28.

Although with a given amount of water vapor in the air, the percentage relative humidity is closely dependent upon the temperature, yet, the two curves in figure 1 giving maximum and minimum temperatures for the same twenty-four-hour period over which the daily evaporation rates were measured, show that there was considerably greater variation in evaporation rates and percentage relative humidity than there was in temperature. This was due to differences in rate of supply of water vapor to the air with any given increase in temperature, whether the water vapor came from air outside the greenhouse or from the soil surface in the greenhouse. A sudden increase in temperature, with a low rate of water vapor supply to the air from the surroundings, was accompanied by a sharp drop in percentage of relative humidity and a rise in the evaporating power of the air. A slow increase in temperature, accompanied by a high rate of water vapor supply from the surroundings, was accompanied by little change in the relative humidity with increase in temperature. Therefore, as far as the relation between the concentration of the nutrient solution to the occurrence of blossom-end rot may be dependent upon the percentage of relative humidity or the evaporating power of the air, so far must temperature remain an *indirect* factor.

## GROWTH AND DRY WEIGHT PRODUCTION OF TOPS

The tomato plants in each of the several series started to grow rapidly, but differences in nutrient supply were soon reflected in the growth responses. Representative plants in each of the several series, are shown in figure 2 as they appeared on September 4, three weeks after the beginning of the nutrient treatments. Plants in series A, C, D, and E, with which the



FIG. 2. Tomato plants on September 4, grown with complete nutrient solutions of different osmotic concentrations. Left to right: series A, 0.08 atm.; series B, 0.08 atm.; series C, 0.44 atm.; series D, 0.83 atm.; series E, 1.70 atm.; series F, 3.10 atm. Nutrient solution of series A supplied at a rate four times that in series B.

nutrient solutions of low and intermediate concentrations were used, started and continued to make relatively more rapid growth than plants in either series B or F. The leaves in the plants of the former series were darker green and larger in size, and the mesophyll of the leaves more savoyed, the plants taller, the stems thicker with more axial sprouts, than was the case of the plants in series B, with the solution of lowest concentration, or in series F, with the solution of highest concentration.

A small amount of injury to the tips of a few leaves of some plants grown with the solutions of the intermediate and higher concentrations appeared following extended periods of cloudy weather. This injury to tissues was not typical wilting, although there was a slight resemblance, but was associated with the fairly high concentrations of ammonium nitrogen in the solutions in these series and its accumulation in these tissues during periods when the assimilation rate of this ion was low (31). Aside from this injury, no other type of specific tissue injury was noted in the plants grown with solutions of high concentrations throughout the experiment.

No significant growth differences associated with the different grades of sand used occurred, and, therefore, further comparisons between series will be made only with reference to the nutrient treatments in the different series.

The differences in growth of the plants in the different series were apparent throughout the experiment. At the time of harvest on September 21 the plants in series A were larger than those in series B and even slightly

greater than those in series C in spite of the fact that plants in series C were grown with a solution of an osmotic concentration more than four times as great, but supplied at one-fourth the rate as the solution in series A. The relatively poor growth of plants in series B was due to a lack of available nutrients, particularly nitrogen, and shows, as has been pointed out by HOAGLAND (10) and JOHNSTON and HOAGLAND (11), that the rate of renewal of solution as well as the initial concentration are both important factors in the relation between the concentration of the nutrient solution and the growth of the plants. The less rapid rate of growth of plants in series F compared with that in series C, D, and E, considered in terms of the relation between the concentration of the solution and nitrogen assimilation of the plants, will not be discussed here in detail. Reference has previously been made to this relation by the writer (20), and also by NIGHTINGALE and FARNHAM (18).

One-half of the plants of each series were harvested on September 21 and figure 3 shows representative plants at this time. Table III gives the



FIG. 3. Tomato plants on September 21, grown with complete nutrient solutions of different osmotic concentrations. Left to right: series A, 0.08 atm.; series B, 0.08 atm.; series C, 0.44 atm.; series D, 0.83 atm.; series E, 1.70 atm.; series F, 3.10 atm. Nutrient solution of series A supplied at a rate four times that in series B.

values for the green weight, dry weight, and the percentage dry weight of various parts of these plants. There was little difference between the total green weights per plant in series C, D, and E—the values being 256, 260.1 and 259.7 gm. respectively. The green weight of series A plants, 228.6 gm., was somewhat lower than these, while the green weight per plant in series B, 122.2 gm., was less than one-half this value. The green weight per plant in series F was 184.2 gm. In general, the dry weights per plant correspond relatively in order of value with the green weights in the respective series. The total dry weight per plant of series E, however, was 29.86 gm., which

TABLE III

AVERAGE GREEN WEIGHT, PERCENTAGE DRY WEIGHT, AND TOTAL AVERAGE DRY WEIGHT PER PLANT OF LEAVES, STEMS, AND FRUITS OF TOMATO PLANTS GROWN WITH SOLUTIONS OF DIFFERENT OSMOTIC CONCENTRATIONS

SERIES	OSMOTIC CONC.	AVERAGE GREEN WEIGHT PER PLANT				PERCENTAGE DRY WEIGHT				TOTAL AVERAGE DRY WEIGHT PER PLANT			
		LEAVES			TOTAL	LEAVES			%	LEAVES			gm.
		gm.	STEMS	FRUITS		gm.	STEMS	FRUITS		gm.	STEMS	FRUITS	
A	atm. 0.08*	130.6	48.3	49.7	228.6	11.43	10.85	7.40	%	14.93	5.26	3.68	23.87
B	0.08	58.5	23.7	40.0	122.2	11.70	10.85	6.73		6.84	2.57	2.69	12.10
C	0.44	160.5	53.4	42.1	256.0	11.27	10.06	8.25		18.09	5.37	3.47	26.93
D	0.83	172.1	59.6	28.4	260.1	10.90	9.41	7.85		18.95	5.61	1.93	26.49
E	1.70	170.8	66.8	22.1	259.7	12.16	10.58	9.14		20.77	7.07	2.02	29.86
F	3.10	115.5	42.0	26.7	184.2	13.00	12.12	10.24		15.02	5.09	2.73	22.84

\* Nutrient solution supplied at a rate four times that in series B.



was greater than the average dry weight of the plants in any of the other series. At this phase of plant development, the fruits on the plants on all series were small and immature, and although the green and dry weights of fruits in series D, E, and F were less than those of series A, B, and C, these differences were reversed as fruit maturity progressed.

The tissues of plants in series F were lower in water content than corresponding tissues in the plants of other series as shown by the percentages of dry weight. For example, the percentage dry weight of the stems of series F was 12.12 as compared with 9.41 for series D, which indicates that growth of the plants in series F was limited by an available water supply for the various tissues, and that the water content of the tissues of these plants decreased with increase in salt concentration of the nutrient solution.

Differences in the water content of ripe, mature fruits grown in the several series were much greater than the differences in the water content of the immature fruits. Table IV gives the percentage of dry weights of

TABLE IV

PERCENTAGE DRY WEIGHT OF GREEN IMMATURE AND RIPE FRUITS HARVESTED ON DECEMBER 4 FROM TOMATO PLANTS GROWN WITH COMPLETE NUTRIENT SOLUTIONS OF DIFFERENT OSMOTIC CONCENTRATIONS

SERIES	OSMOTIC CONC.	FRUITS	
		PERCENTAGE DRY WEIGHT	
		GREEN	RIPE
	<i>atm.</i>	%	%
A. ....	0.08*	.....	4.54
B. ....	0.08	.....	4.71
C. ....	0.44	7.12	6.69
D. ....	0.83	6.45	7.11
E. ....	1.70	7.58	8.44
F. ....	3.10	10.65	..

\* Nutrient solution supplied at a rate four times that of series B.

green fruits and of ripe fruits harvested on December 4 from each series. Only normal fruits with no blossom-end rot are here represented. Where no values appear in the table, fruits of that series were unavailable. The percentage dry weights of ripe fruits of series A and B were 4.54 and 4.71 respectively, whereas the percentage dry weight of ripe fruits in series E was 8.44, or nearly twice as great, and reflects the great difference in the availability of water supply for these respective tissues.

These values of green and dry weight production show that in sand culture with nutrient solutions of 0.44, 0.83 and 1.7 atmospheres, tomato plants made excellent growth; that with a nutrient solution having an osmotic value of 0.08 atmospheres, good growth resulted only when the nutrient solution was supplied at a rate sufficiently rapid to furnish an adequate nutrient supply and that although good growth resulted with a nutrient solution of 3.1 atmospheres osmotic value, growth of these plants was limited by factors other than mere quantitative nutrient supply. The relation between the water content of the tissues and the osmotic value of the extracted juices will be discussed later.

#### ROOT DEVELOPMENT

The roots of the plants in the several series presented distinct differences in appearance. The roots of the plants grown with the solution of highest concentration were more slender and much more wiry than the roots of the plants in the other series. When bent double, these roots did not break readily as did those of the plants grown with the solution of the lowest concentration. The roots apparently had matured early before much increase in diameter had taken place. Many of these roots in the series F plants were light brown in color, except toward the tips which were white, indicating early suberization of root tissues. The individual roots of the series F plants were not as long as those of the series A and B plants. The roots of series A and B plants were brittle, and usually broke when bent double. They were a uniform light yellow to white in color, and were maturing relatively slowly. Each root was larger in diameter and longer than those of series F, giving the root system a coarser and more extensive appearance. The roots grown with the solution of intermediate concentrations, namely, series C, D, and E, were intermediate, in condition and appearance, between the roots of the plants grown with the solutions of highest and lowest concentration. Roots of series C and D plants were excellent, and typical of roots produced under optimum sand culture conditions.

The differences in root structure noted in the several series offer an additional explanation for the observed decrease in water absorption and water requirements of plants grown in solutions of high salt concentration. When the roots mature rapidly with early suberization of epidermal cells, the proportion of active absorbing surface to total root surface is relatively low, and the rate of water absorption, by tissue which has thus matured, is minimized. The differences in roots of these plants grown with solutions of different concentration were similar to the differences in the roots of sweet pea which have been described by NIGHTINGALE and FARNHAM (18).

The possible importance in the several series of the effects of certain ions, due to their specific nature, and aside from their colligative osmotic proper-

ties—for example, calcium and phosphate, on the rate of maturity of and the absorption of water by the root tissues—is not to be here disregarded, but has not been completely evaluated in this experiment.

#### TURGOR AND GUTTATION

Soon after the beginning of this experiment, there was apparent a difference in the internal water relations of the plants of the several series. Although there was no pronounced and typical wilting of the leaves of any of the plants during the course of the experiment, differences in turgor of the tissues were evident. The mesophyll cells in the leaves of plants in series A, B, C, and D, grown with the solutions of lower concentrations, were glistening and shiny in appearance, which is typical of cells with high turgor. Similar leaf tissue cells in series E and F lacked somewhat this glistening, shiny appearance. The leaves of series E and F with the solution of highest concentration did not have as high turgor as did those of the other series. The younger leaves of the plants in these latter series, during the middle of the day when the evaporation rate was highest, dropped slightly more than at any other times of the day. The leaves on plants in series A to D appeared to have maximum turgor at all times of the day.

The appearance of guttation on the plants of some series, and not on others, gave further evidence of differences in turgor. Drops of guttated liquid occurred on the leaves of plants in series A and D during periods of very low transpiration, on September 3, 4, 7, 8, 10, and 14. As indicated in figure 1, the percentage relative humidity of the air was very high on these days, namely, between 85 and 95. Guttation was not observed on any of the plants in any of the series when the relative humidity was much below 85 per cent. and during the last two months of the experiment this value was recorded only twice.

Figure 4 shows a plant on August 25, when the relative humidity was 95 per cent., from series B with large drops of guttated liquid on the tips and margins of the leaves. At no time did guttation take place from the leaves of any plants grown in series E and F, with the solutions of high concentration.

There was considerable difference in the relative amount of guttation in the several series. Practically all the leaves of plants in series A and B showed copious guttation. Guttation was not so abundant from the leaves on series C plants, although most leaves of the plant showed some. On series D, however, under the same conditions, only the younger leaves showed this phenomenon. Therefore, under conditions of high humidity where transpiration rates were low, positive internal pressures, resulting in guttation, occurred with the solutions of the lower concentration used. With solutions of the higher concentrations, however, such great positive pressures did not

occur. Likewise in the same plant turgor differences made evident by differential guttation were maintained in the cells of the respective tissues by differences in either osmotic or imbibitional forces, or a combination of both. The importance of the maintenance of such differences in pressure of the hydrostatic system of different tissues to the incidence of blossom-end rot of the fruits will be discussed later.



FIG. 4. Tomato plant grown with nutrient solution of low osmotic concentration (0.08 atm.) showing drops of guttated liquid on leaves.

#### FRUIT DEVELOPMENT AND INCIDENCE OF BLOSSOM-END ROT

The most striking external differences in the growth of plants in the several series were in the development of fruits, and the incidence of blossom-end rot. These differences were caused primarily by the same factor—namely, relative differences in the adequacy of the water supply for fruit development. The lower relative water requirements of plants grown with solutions of high concentrations than with low concentrations is known (16, 22). Plants in all series produced fertile blossoms which set fruit freely. Figure 2 shows the plants as they came into bloom. Fruits set slightly earlier on plants with the solutions of low concentration as may be seen in table V, but at the time of the first harvest on September 21, plants in all series bore small fruits. Individual fruits in all series developed at approximately the same rate until they reached a size of from 2.5 to 3.5 cm. in diameter. Up to this phase of development, as shown in figure 3, blossom-end rot did not occur in any of the series, with two exceptions, but from this

TABLE V

NUMBER OF FRUITS SET PER PLANT IN EACH SERIES OF CULTURES GROWN WITH NUTRIENT SOLUTIONS OF DIFFERENT OSMOTIC CONCENTRATIONS ON THREE DATES DURING EARLY PART OF EXPERIMENT

SERIES	OSMOTIC CONC.	FRUITS SET PER PLANT		
		SEPTEMBER		
		13	17	21
	<i>atm.</i>			
A	0.08*	1.0	2.4	3.0
B	0.08	0.6	2.0	2.3
C	0.44	1.1	2.6	3.3
D	0.83	1.0	2.0	2.3
E	1.70	0.5	2.9	- 4.8
F	3.10	0.4	3.0	4.3

\* Nutrient solution supplied at a rate four times that of series B.

point on fruits in series E and F increased in size much more slowly, and under favoring conditions developed blossom-end rot much more rapidly than did the fruits in other series. Figure 5 shows fruits chosen on October 14 at a somewhat similar size, namely, 4.7 to 5.7 cm. When environmental

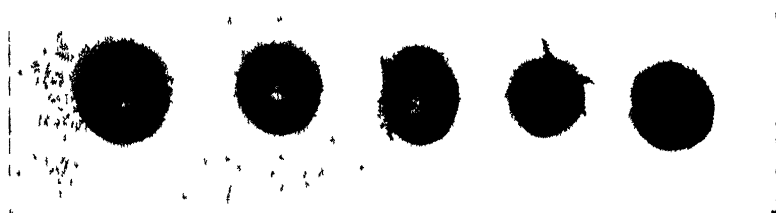


FIG. 5. Immature fruits on October 14, grown with complete nutrient solutions of different osmotic concentrations. Left to right: series A, 0.08 atm.; series C, 0.44 atm.; series D, 0.83 atm.; series E, 1.70 atm.; series F, 3.10 atm. Fruits from series E and F exhibit blossom-end rot.

conditions favored the development of blossom-end rot, the initial symptoms of this disease appeared more quickly on fruits of this size and phase of maturity, than on either the smaller less mature, or on the larger more mature fruits. Maturity, or the character and composition of the fruits, rather than the size itself, is the important factor in this relation, and this will be discussed in connection with the ability of the affected tissues to obtain and retain an adequate water supply for tissue development.

The difference in fruit size of the several series continued to be greater until the fruits ripened. Table VI shows the average weight of individual ripe fruits in the several series on several harvest dates, for both normal fruits and those exhibiting blossom-end rot injury. The average weight

TABLE VI

AVERAGE WEIGHT PER RIPE FRUIT ON EACH OF THREE HARVEST DATES, TOTAL FINAL AVERAGE WEIGHT PER FRUIT, TOTAL NUMBER OF FRUITS PER PLANT, AND PERCENTAGES OF TOTAL NUMBER OF FRUITS WITH BLOSSOM-END ROT FROM PLANTS GROWN WITH NUTRIENT SOLUTIONS OF DIFFERENT OSMOTIC CONCENTRATION

NUTRIENT TREATMENT		AVERAGE WEIGHT PER FRUIT			SUMMARY		
SERIES	OSMOTIC CONC.	Nov. 4	Nov. 19	Dec. 4	TOTAL FRUITS PER PLANT	AV. WT. PER FRUIT	PER-CENTAGE OF TOTAL FRUITS
Normal fruits							
	<i>atm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>		<i>gm.</i>	<i>%</i>
A .....	0.08*	140	129	111	5.5	127	100
B .....	0.08	.....	87	51	3.0	69	100
C .....	0.44	105	82	79	2.5	89	83
D .....	0.83	114	117	44	3.8	92	72
E .....	1.70	.....	38	47	3.3	43	23
F .....	3.10	.....	25	10	0.5	18	20
Fruits with blossom-end rot							
A .....	0.08*	.....	.....	.....	0.0	.....	0
B .....	0.08	.....	.....	.....	0.0	.....	0
C .....	0.44	27	44	.....	0.5	36	17
D .....	0.83	91	.....	70	1.3	81	28
E .....	1.70	54	.....	57	2.5	56	77
F .....	3.10	26	58	31	2.0	38	80

\* Nutrient solution supplied at a rate four times that in series B.

per ripe normal fruit varied from 127 gm. in series A, to 18 gm. in series F. The small size of the few fruits in series F, which ripened normally, was partly due to imperfect fruit development caused by an occasional incomplete pollination of the original blossoms. Fruits in series F, which developed from completely pollinated blossoms, were somewhere larger and averaged 38 gm. in weight when ripe, but these fruits invariably developed blossom-end rot. Figure 6 shows the fruits on a plant from series A, on November 4. Although the ripe fruits harvested from plants in this series,



FIG. 6. Representative tomato plant from series A, grown with complete nutrient solution of 0.08 atm osmotic value. The ripe fruits are of large size and show no blossom end rot.

at this time, averaged 140 gm., the largest fruits weighing less than 200 gm., yet, one of these fruits, shown on the lower cluster in figure 6, when harvested later weighed 400 gm., an unusual size for a fruit of this variety. Figure 7 illustrates typical fruits from each series as they appeared when ripe. The fruit from series A on the left was 7.4 cm. in diameter; that from series F, showing blossom-end rot, was 3.6 cm. in diameter. The two fruits on the

right show the typically shrunken appearance of ripe fruits with blossom-end rot. The fruits in series B, and to a slight extent in series A, showed, as did the growth of the plants themselves, the effect of a somewhat limited nitrogen supply. These latter fruits were somewhat more irregular in outline and less globular in shape than fruits in series C and D, and the flesh of the fruit around the stem of many showed a slightly yellow tinge of color as the fruits ripened.

As shown in table VI, blossom-end rot occurred on most fruits grown with solutions of high concentration and did not occur on solutions of the lowest concentration used. The percentage of total fruits with blossom-end rot was: series F, 80 per cent; series E, 77 per cent., series D, 28 per cent.; and series C, 17 per cent. In series A and B there was no blossom-end rot. The only fruits in series F which failed to develop blossom-end rot were the occasional very small fruits averaging 18 gm. in weight, which, as was previously

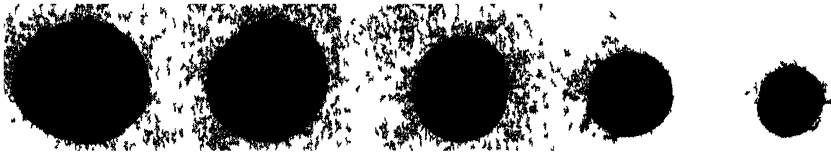


FIG. 7. Ripe fruits on November 4, grown with complete nutrient solutions of different osmotic concentrations. Left to right: series A, 0.08 atm; series C, 0.44 atm; series D, 0.83 atm; series E, 1.70 atm.; series F, 3.10 atm. Fruit from series E and F show blossom end rot in advanced stages.

indicated, never reached the size when blossom-end rot was found to most readily develop.

The initial symptoms of blossom-end rot appeared most often during periods of bright, warm weather, accompanied by high evaporation rates, which followed periods of cool, dark weather accompanied by low evaporation rates. The initial symptoms were externally apparent in a very few days after such a sudden change in these environmental factors. The differences in evaporation rates of the air by their direct effect upon the water relations of the plant appeared to be the principal factor influencing the development of blossom-end rot, rather than the indirect factors of light and temperature. Such changes in evaporation rates occurred frequently during the experiment. Figure 5 shows green fruits in series E and F with blossom-end rot as they appeared on October 14, which was the third day of an extended period of relatively high evaporation rates (fig. 1) following two periods of low evaporation rates,—one on October 7 and one on October 11.

After blossom-end rot had seriously involved a considerable portion of the tissues, fruits rarely ripened normally thereafter. When conditions



associated with the occurrence of blossom-end rot recurred repeatedly, the affected part of the fruit increased in size and involved a large portion of the fruit. Figure 8 shows two stages in the advance of this disease to a point



FIG. 8. Tomato fruits grown in sand culture. Left, normal fruit from series A; right, three fruits from series F showing blossom-end rot in several stages of development.

where recovery did not take place. When, however, the conditions favoring the development of blossom-end rot, did not reoccur before fruit maturity, an occasional fruit, such as the one on the right in figure 8, ripened with no further injury beyond the slight browning of the outer epidermal tissue which constituted the first visible symptoms of the occurrence of this disease.

In this experiment both nitrate and ammonium were used as sources of nitrogen. In view of the repeated references to the greater incidence of blossom-end rot where ammonium nitrogen was used, than where nitrate nitrogen was used, it may be wondered whether the form of nitrogen supplied in the nutrient solution was an important direct factor associated with the development of this disease. Comparisons of the effect of the two forms of nitrogen on the rate of incidence of blossom-end rot were not made in this experiment. But in preliminary experiments, under almost the identical cultural, seasonal, and environmental conditions which obtained during a part of this experiment, and where the nutrient solutions contained nitrate as the sole source of nitrogen, almost all of the fruits developed blossom-end rot when the osmotic concentration of the solution used was 1.75 atmospheres. When the osmotic value was decreased to one atmosphere, keeping the proportion of the salts the same, not a single fruit developed blossom-end rot thereafter. The development of blossom-end rot is not necessarily to be associated solely with either ammonium or nitrate nitrogen. Salts containing either form of nitrogen if present in high concentrations obviously exert an osmotic effect upon the absorption of water. However, aside from the osmotic effect concerned, if the use of nitrogen in high concentration in any form which can easily be assimilated results in an extremely vegetative growth of a tomato plant, such a plant will have a small proportionate root system, a low proportionate amount of water conducting tissue, thin cell

walls, and other features (19) which, under conditions of high evaporation, make it wilt more quickly than a plant grown with a relatively low rate of nitrogen assimilation. Fruits on such a plant are relatively more susceptible to the development of blossom-end rot than are those of the low nitrogen plant. The great relative rate of incidence of blossom-end rot of the fruits of vigorously vegetative plants is a matter of common observation and has been referred to.

#### OSMOTIC VALUES OF EXPRESSED TISSUE JUICES AND WATER SUPPLY OF FRUITS

In order to account for the loss of water from the fruits which occurs during the development of blossom-end rot, determination of the osmotic values of expressed tissue juices of fruits and young stems and leaves of the plants in the several series were made. Table VII gives these values. The osmotic values of the fruit tissues ranged from 6.68 atmospheres in series A and C to 10.79 atmospheres in series F, and for the young stem and leaf tissue from 8.19 atmospheres in series B to 12.99 atmospheres in series F. There is thus a difference of over 4 atmospheres between the osmotic values of the expressed juices of analogous tissues of plants grown with solutions of the lowest and highest concentrations. These differences in osmotic values show that there exist great differences in the availability of water in the tissues of these plants in the several series. There were also differences in the actual water content of the stem tissue extract in the several series, as determined by the refractometer, ranging from 94.55 per cent. for series A to 92.55 per cent. for series F. The small differences in water content, compared with the total percentage assume a greater significance when the percentage of total solids, which include solids of all degree of osmotic activity, are compared. These values vary from 5.45 per cent. in the case of series A to 7.45 per cent. in series F, representing a difference of 37 per cent. over the series A value. The relative *osmotic* values of the fruit and stem tissue extracts in the several series, taking the respective values in series A as 100, are also given in table VII. The relative osmotic values of the series F plants are 162 for the fruit and 155 for the stem. In the cases of series D, E, and F, at least, it may be seen that the relative osmotic values of the fruits and of the stems are higher than the relative values for the percentage of total solids of the stems. This shows that there was a higher proportion of osmotically active solids in the tissues of the plants in these three series and emphasizes the probable importance of the inorganic salts in the osmotic relations of these tissues.

In each series the osmotic values of the extracts of the stem and leaf tissue exceeded those of the fruit tissue. These differences ranged in magnitude from 1.62 atmospheres in series A to 3.63 atmospheres in series C, and indi-

**TABLE VII**  
**OSMOTIC VALUES OF TISSUE EXTRACTS OF SMALL GREEN FRUITS AND YOUNG STEMS, AND PERCENTAGE OF WATER AND SOLIDS OF THE EXTRACT OF STEMS OF TOMATO PLANTS GROWN WITH COMPLETE NUTRIENT SOLUTIONS OF DIFFERENT OSMOTIC CONCENTRATIONS**

NUTRIENT TREATMENT		TISSUE EXTRACT						
		OSMOTIC VALUES					PER- CENTAGE OF WATER	PERCENT- AGE OF SOLIDS
		FRUIT	STEM	RELATIVE VALUES SERIES A = 100		GRADIENT BETWEEN FRUIT AND STEM	STEM	STEM
				FRUIT	STEM			
SERIES	OSMOTIC CONC.							RELATIVE VALUES SERIES A = 100
A	atm. 0.08*	atm. 6.68	atm. 8.30	100	100	atm. 1.62	% 94.55	% 5.45
B	0.08		8.19		99		94.40	5.60
C	0.44	6.68	10.31	100	124	3.63	94.25	5.75
D	0.83	7.71	10.51	115	127	2.80	94.00	6.00
E	1.70	9.17	12.21	137	147	3.04	93.85	6.15
F	3.10	10.79	12.99	162	155	2.20	92.55	7.45

\* Nutrient solution supplied at a rate four times that in series B.

cate that a water deficit must surely exist in the tissues of the fruit and that under conditions of high transpiration rates, not only did a continued water supply become unavailable to the tissues of the fruit, but actual withdrawal of water took place from these tissues. In plants in series E and F particularly, this loss of water from the tissues of the fruits was great enough to bring about the tissue desiccation and cell collapse known as blossom-end rot.

These results are in agreement with those of HAAS and KLOTZ (8), who report higher osmotic values for the extracted sap of lemon leaves than of the fruits with attendant loss of water from the fruits; with those of BARTHOLOMEW (2), who by dendrographic measurements found actual water loss from lemon fruits; and with MACDOUGAL (14), who by similar measurements reported loss of water from tomato fruits.

It has been assumed that during the development of blossom-end rot the principal loss of water from the fruits took place through the pedicel, rather than from the surface of the fruit. This apparently was true since fruits of series F in a state of maturity, such that blossom-end rot developed rapidly under favoring conditions if allowed to remain attached to the plant, lost water much less rapidly after removal from the plant than when still attached, and after removal from the plant did not develop blossom-end rot. Transpirational loss, therefore, from the fruit surface was concluded to be negligible compared with the loss through the pedicel to the stems and leaves.

Some explanation must account for the fact that severe blossom-end rot developed in fruits of series F plants when the determined osmotic gradient between fruits and stems was found to be 2.20 atmospheres whereas this disease did not develop in fruits in series A plants when the osmotic gradient between fruits and stems was 1.62 atmospheres, only slightly less. The osmotic gradients between fruits and stem tissue in the plants of the other series was even greater than either of these two differences. It is significant that the smallest gradient occurred in series A plants where blossom-end rot did not develop; and this alone may primarily account for the phenomenon. However, an additional explanation seems pertinent. The existence of an osmotic gradient between two tissues does not *per se* necessarily imply movement of water. The difference in osmotic pressure, which might otherwise be expected to result in water movement, can be counteracted by imbibitional forces establishing a gradient in the opposite direction. No movement of water then takes place and the tissues retain their supply against a determined osmotic gradient.

This relation between the osmotic and imbibitional forces of fruit tissue on the one hand, and leaf and stem tissue on the other hand, apparently obtained in series A and B. Moreover, accompanying a considerable variation of osmotic concentration of cell sap which is known to occur periodically (32), cells, although losing a slight amount of water at certain times of the

day, may yet recover without serious injury when water is again available, provided loss of water does not continue to take place too long. Any loss of water from fruits of series A and B plants, which may have occurred, was undoubtedly very slight and of short duration owing to the low resistance to water absorption which the solution of low concentration used with these series offered. Loss of water from the tissues of fruits in series C, D, E, and F, on the other hand, took place not only more rapidly as indicated by the greater osmotic gradients, but undoubtedly this loss of water occurred over a longer period of time owing to the higher resistance to water absorption which these solutions offered as compared with the nutrient solution used with series A and B.

The notably high imbibitional forces of young cells (5, 24, 25, 26), often exceeding in value the osmotic forces, undoubtedly accounts for the fact that very young, actively growing tomato fruits did not develop blossom-end rot even under the conditions of high transpiration intensities, and the severe fluctuations in evaporation rates which occurred at certain times during this experiment.

During the development of blossom-end rot there is, in addition to a loss of water from the fruit as a whole, competition for water between different tissues of the same fruit. By the refractometer method, it was found that differences in water content of the extracts of various tissues of the same fruit did exist. The water content of the wall tissue near the stem end of the fruit was 93.05 per cent. whereas the water content of the tissue of the wall near the blossom-end of the fruit was 93.6 per cent. The water content of the juice of the seed cavity was still higher – 93.85 per cent. Although differences in water contents do not necessarily prove that attendant differences in osmotic values exist, yet, the close inverse relation between water content and osmotic value of plant tissues is very common as STODDART (27) has shown.

#### GAIN OF WATER BY FRUITS

Whereas, associated with the development of blossom-end rot, tomato fruits on plants grown with nutrient solutions of high concentration lost water during periods of high transpiration, under the reverse environmental conditions, when grown with solutions of low concentration and under low transpiration, fruits gained water. The relatively high water content of the fruits in series A and B has been noted. The gain in water by fruits following sudden change of environmental conditions may be so rapid that actual tissue rupture takes place. Such cracking is very common in some varieties of tomatoes in the field (6), when they are almost ripe, particularly during a rain which follows a period of bright warm weather. Such cracking or splitting of the fruits usually occurs coincidentally with guttation from the

leaves, under conditions of very low transpiration. In this experiment, although there was an occasional fruit in series A which showed this splitting, environmental conditions were not particularly favorable to the occurrence of this cracking during the latter part of the experiment when the fruits were ripening. However, such splitting of nearly ripe fruits has occurred at other times repeatedly with solutions of low concentration during a cloudy period of very high humidity immediately following a period of bright weather. There was no splitting whatever of the fruits in series C, D, E, or F. Furthermore, whereas blossom-end rot develops principally on green fruits, cracking occurs largely with ripe or nearly ripe fruits which are high in sugars.

A mechanism of tissue rupture in Stayman apples, which appears to be similar to that involved in cracking of tomato fruits, has been reported by VERNER (34) to occur under a combination of environmental conditions just the reverse of those which, in this experiment, were found to be associated with the development of blossom-end rot of tomato fruit. Cracking of the external tissue of the apple occurred when underlying and adjacent fruit tissue had a high osmotic value with reference to the surrounding tissue, but this cracking took place only under conditions of high humidity. Under these conditions of low relative transpiration, water in the plant became more available and moved into the tissues concerned. This reported injury to the apple tissue occurred usually during or following a rain and therefore there undoubtedly was a coincidental decrease in the salt concentration of the soil solution, although these values were not reported. As VERNER pointed out, however, the incidence of the injury was not solely correlated with any particular water content of the soil.

#### MODIFYING FACTORS

Factors of the environment which more or less directly affect the water supply of the diseased tissue of the tomato fruit may modify the relation between the concentration of the nutrient solution and the development of blossom-end rot.

First, the rate of change of nutrient solution is an important factor. A rapid movement and abundant supply of solution in the substrate tends both to minimize the rate of change in salt concentration of the absorbing film of the roots and also to keep to a minimum the attractive force of the substrate particles for water—both factors of which affect the absorption of water by the roots. In this experiment the rate of nutrient solution supply was the same in all the series except series A, where the rate of supply was four times that of the other series. Although two slightly different sized sand particles in the substrate were used, there was no apparent difference in the incidence or rate of development of blossom-end rot of the fruits grown with the grades

of sand used. There was undoubtedly in the cultures of each series some variation in the concentration of the absorbing films and in the attractive force of the particles of the substrate for water under the varying rates of transpiration, but such changes are not considered the principal limiting factors in these experiments.

Secondly, since the pH value of the nutrient solution affects directly or indirectly the absorption of water by the plant, this factor may also become important in the relation between the concentration of the solution and the incidence and development of blossom-end rot of the tomato fruit. The original pH values of the different nutrient solutions herein employed were largely dependent upon the concentration of the phosphate ion, reference to which, with regard to the growth of roots, has already been made. Table VIII gives the pH values of the solutions in each series before and after

TABLE VIII

AVERAGE pH VALUES OF EXCESS SOLUTIONS ESCAPING FROM THE CULTURES IN EACH SERIES RECEIVING NUTRIENT SOLUTIONS OF DIFFERENT OSMOTIC CONCENTRATIONS, AS COMPARED WITH INITIAL pH VALUES OF EACH SOLUTION

SERIES	OSMOTIC CONC.	pH VALUE OF NUTRIENT SOLUTIONS			
		INITIAL	FINAL		
			SEPT. 2	SEPT. 14	NOV. 21
	<i>atm.</i>	<i>pH</i>	<i>pH</i>	<i>pH</i>	<i>pH</i>
A .....	0.08	5.2	5.3	5.5	4.2
B .....	0.08	5.2	5.4	5.7	4.0
C .....	0.44	5.0	5.2	5.2	4.1
D .....	0.83	4.9	4.9	5.1	4.1
E .....	1.70	4.8	4.4	4.5	4.1
F .....	3.10	4.7	4.4	4.1	4.1

passing through the cultures. Previous to the time of the first harvest on September 21, the pH values of the escaping solutions in series A, B, and C were each slightly higher than they were initially in the respective series. The pH values of the solutions escaping from the cultures in series E and F were lower than they were initially. During the latter part of the experiment, however, when distinct differences in the rate of incidence of blossom-end rot in the several series occurred, the solutions escaping from cultures in all series were approximately the same in pH value, namely, 4 to 4.2. Therefore, in this experiment observed differences in the pH value of the solutions in the several series are not in themselves considered principal limiting factors in the relation between the salt concentration of the nutrient solution and the incidence and rate of development of blossom-end rot.

Thirdly, the decrease in the rate of water absorption, which is associated with a low available oxygen supply to the roots (16) and the increase in suction pressure of the root cells attendant with low oxygen supply (33) may become a very important factor in affecting the development of blossom-end rot of the tomatoes grown in nutrient solutions. Thus, this disease of the fruit has been produced in this laboratory when the plants were grown in nutrient solutions of approximately only one-half atmosphere osmotic value but when insufficient aeration of the culture solution was provided.

The external factors which directly or indirectly affect transpiration, such as light intensity, the humidity, temperature, and rate of movement of the surrounding air, are likewise important factors in the development of blossom-end rot. The extent of regulation of transpiration by stomatal movement incident to changes in light intensity was not recorded in this experiment, but the importance of such regulation is not to be underestimated. The close association between changes in light intensity and the development of blossom-end rot in this experiment has, however, been previously noted, as well as the reported decrease in the rate of incidence of this disease on the fruits of shaded plants grown in soil compared with unshaded plants. The effect of the degree of humidity and temperature have been discussed, but the importance of the rate of air movement upon the development of blossom-end rot through the indirect effect upon the transpiration intensity should also be emphasized.

Before concluding the discussion of the relation between the solute concentration of the nutrient solution and the incidence of blossom-end rot of the fruit, the significance of wide variation in the magnitude of the saturation deficit of the tissues should be re-emphasized. Whereas, rapid growth of all tissues is coincident with relatively *low* saturation deficits, the occurrence of blossom-end rot must be coincident with *high* saturation deficits of the cells concerned. With culture solutions of high salt concentrations, the relatively high resistance to water absorption by the plants at a rate rapid enough to replace transpiration loss increased the magnitude of the saturation deficits of these plant tissues when the rate of transpiration was high, as compared with that present in these same tissues at other times when the transpiration rate was much lower. Thus, whereas water was available for the growth of fruits when the transpiration rates were relatively low, yet withdrawal of water from the affected tissues of the fruit took place when the transpiration rates were relatively high. Whether blossom-end rot would develop under conditions of constant low evaporation rates if the tomato plants were grown with solutions of high concentration, was not determined.



### Conclusions

The close dependence of the plant upon an abundantly available water supply for the rapid growth of tissues and for the development of fruit is emphasized.

Rapid development of vegetative tissues and fruit of the tomato is facilitated with nutrient solutions of low concentration provided an abundant supply of the solution is available. With solutions of high salt concentrations, growth of the tissues may be limited by lack of available water.

A concomitant result of this lack of a readily available water supply for the tissues of the plant, is the development of blossom-end rot of the fruit which occurs to a high degree when nutrient solutions of high salt concentrations are employed.

Any factor which either seriously restricts the rate of absorption or greatly increases the rate of transpiration of water by the plant will increase the probability of the incidence and development of blossom-end rot of the fruit.

### Summary

1. Tomato plants from a selected strain of the Marglobe variety were grown in sand culture in the greenhouse with nutrient solutions of five different osmotic concentrations; namely, 0.08, 0.44, 0.83, 1.7, and 3.1 atmospheres. A comparison was made of the effects on growth of two rates of supply of the solution of lowest concentration.

2. A deficiency of nutrients, especially of nitrogen, limited growth in solutions of 0.08 atmosphere osmotic value supplied at the rate of one liter per plant per day. At the higher rate of supply of the same solution of four liters per plant per day, plants made very good vegetative growth.

3. With nutrient solutions of 0.44, 0.83 and 1.7 atmospheres osmotic value, plants made excellent vegetative growth and had greater total green and dry weight per plant than those in solutions of lowest and highest osmotic value.

4. Vegetative growth of plants with solutions of 3.1 atmospheres osmotic value was good, but was limited by factors other than quantitative inorganic nutrient supply, the most important of which was apparently low available water for tissue development.

5. Fruits set freely on plants grown with nutrient solutions of each concentration.

6. Tomato fruits produced on plants grown with the nutrient solutions of 3.1 atmospheres osmotic value were very much smaller than those produced on the plants grown with the solutions of lower concentration. Many fruits grown with solutions of 0.08 atmosphere were of exceptionally large size.

7. The roots of plants grown with solutions of 0.08 atmosphere osmotic value were brittle, succulent, relatively large in diameter, almost wholly white in color, and appeared to be maturing slowly. The roots of plants grown with the solution of 3.1 atmospheres osmotic value were tougher, less succulent, smaller in diameter, slightly more creamy yellow to brown in color, and appeared to be maturing more rapidly than the roots of the plants grown with the solution of lowest concentration.

8. There was a pronounced difference in the internal water relations of plants grown with the nutrient solutions of different concentrations as evidenced by differences in guttation from the leaves. Under conditions of low transpiration guttation took place abundantly from the leaves of plants grown with solutions of the lowest concentration used, but not at all from the leaves of plants grown with the solutions of the two highest concentrations.

9. The percentage dry weights of fruit, stems, and leaves varied with the concentration of the solutions, the values being the highest in the solution of 3.1 atmospheres osmotic value and lowest in solutions of 0.08 atmosphere.

10. Approximately 80 per cent. of the fruits on plants grown with solutions of the two highest concentrations employed developed the physiological tissue disorder known as blossom-end rot. This disorder consists of the turning brown, shrinking, and death of the tissues at the styler or blossom end of the fruit accompanying the loss of water from these tissues.

11. No blossom-end rot occurred on fruits grown with the solution of the lowest concentration employed.

12. The development of blossom-end rot was associated with wide fluctuations in the rates of transpiration as indicated by fluctuations in the rates of evaporation and occurred during a period of high transpiration intensities.

13. A slight amount of cracking of fruits occurred under conditions of low transpiration intensities but only in the case of plants grown with the solution of the lowest concentration.

14. There was a difference of approximately four atmospheres between the osmotic values of the extracted juices of similar tissues of plants grown with solutions of lowest and highest concentrations, these values ranging from 6.68 to 10.79 atmospheres in the case of the fruits and from 8.19 to 12.99 atmospheres in the case of the stems.

15. An osmotic gradient of 1.62 to 3.63 atmospheres was found to exist between the extracted juices of the tissues of the fruits and those of the stems and leaves, in the plants grown with solutions of the various concentrations employed. The smallest osmotic gradient occurred in the plants grown with the solutions of lowest concentration.

16. The significance of the differences in osmotic and imbibitional pressures of fruit, and stem and leaf tissues of the plants in the various series

in relation to the incidence and development of blossom-end rot of the fruit is discussed.

17. The importance of the factors of light, temperature, humidity, rate of air movement, and the pH value and oxygen tension of the solution to the development of blossom-end rot of the fruit in solutions of different nutrient salt concentrations are either noted or discussed.

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# THE FROST-HARDENING MECHANISM OF PLANT CELLS<sup>1</sup>

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(WITH THREE FIGURES)

## Introduction

In the foregoing papers of this series (25, 26) we have given an account of our more intensive researches on the physiology of cold resistance, approached always through a study of living cells. We now describe a number of lesser excursions into the same field, and combine with this a survey of the whole problem of hardening in the light of those changes which have been found to accompany it. The relation of proved hardening changes to the mechanism of resistance must remain hypothetical unless we know the type of injury which has to be resisted. This, however, is still a problem, and evidently a complex one. The immediate cause of death is not always the same. Sometimes it is only indirectly related to temperature, as in soil heaving, smothering by ice, and physiological drought. Often there is a time factor which would seem to involve a mechanism different from that responsible for immediate killing.

Though we confine our attention to the more direct and immediate action of frost, the problem is still complicated because, as we shall see, the mode of injury varies with conditions, such as the rate of freezing or the rate of thawing, and also with the type of plant. Very tender plants are killed merely by chilling to temperatures which are still above the freezing point of their juices, or even above 0° C., but most plants of temperate regions suffer no harm unless ice forms in their tissues, and they may be supercooled with impunity. The well-known resistance of dry seeds and spores to the extreme cold produced by liquid air or liquid hydrogen shows that low temperature *per se* is not fatal. There is also some evidence, though rather indirect, that the amount of injury to a particular tissue is more or less proportional to the amount of ice formed in it (1, 31). Whatever the mechanism of frost injury, apparently any change which reduces or prevents ice-formation will have a hardening effect, and certain theories of hardening are based entirely on this type of resistance. But tissues do freeze, and the major problem before us is how hardening enables a plant to endure an amount of freezing that is fatal in the unhardened state. It is this problem that depends on the mechanism of injury for its solution, and it will therefore be discussed in relation to theories of the same. These fall naturally into two main groups: Those that regard injury as an effect

<sup>1</sup> Investigation carried out with financial aid of the National Research Council of Canada. This is the third of a series of papers on frost-hardening processes.

of dehydration of the cells; and those that regard it as mechanical. It is convenient, therefore, to classify hardening changes, according to the type of resistance which they seem to offer, into the following categories:

- I. Resistance to formation of ice.
- II. Resistance to dehydration effects of ice formation.
- III. Resistance to mechanical effects of freezing and of thawing.

#### I. Resistance to formation of ice in tissues

The factors tending to prevent or reduce freezing which are found to become more active with hardening are supercooling, depression of the freezing point, and reduction in the amount of free or freezable water.

#### SUPERCOOLING

Supercooling is always observed when tissues are exposed to freezing temperatures, and has been found (at least in insects) to be greater in hardened than in unhardened tissues (40). Generally, however, the maximum extent of the supercooling is but a few degrees, and its duration brief. In such cases it can act only as a first line of defense. But cases of greater and more prolonged supercooling—or pseudo-supercooling—are also on record. WIEGAND (47) found that in some trees the buds are not frozen at  $-18^{\circ}\text{C}$ ., and in a smaller number even at  $-26^{\circ}\text{C}$ ., more than  $20^{\circ}$  below the freezing point of their cell sap. LEWIS and TUTTLE (27) found that living leaves of *Pyrola* froze at a temperature  $28.5^{\circ}\text{C}$ . lower than that required to freeze dead leaves. ILJIN (21) says that on account of supercooling, leaves of evergreens, such as *Hedera helix*, often resist temperatures reaching below  $-20^{\circ}\text{C}$ . in Central Europe.

In a plant, the conditions for supercooling are well fulfilled as regards breaking up of the liquid mass into separate droplets and capillary columns; but it would seem to be essential for the continuation of the state that the cells be isolated by ice-proof barriers which will prevent spread of crystallization beyond any locus in which it may chance to originate. For this to be maintained there must be very little freezable water in the walls.

In this connection it is significant that reduction of water content (which practical men call "maturing") is a regular feature of hardening. The percentage reduction is not usually great, but a small reduction of cell volume can greatly reduce the turgor pressure of the cells and correspondingly greatly increase their suction tension, which of course reduces the water in their walls. Thus, the water which remains in the walls is far below saturation point, and its menisci are retracted into the ultra-microscopic pores, a condition which tends to prevent ice formation. Also, it is known that wilting increases frost resistance, and the presence of free intercellular moisture decreases it. But hardy plants, and animals also, do not

depend upon supercooling for their protection. At a sufficiently low temperature, ice always forms, except in dry objects like seeds, and tissues may be frozen till brittle without fatal effect. In such cases, toleration rather than prevention of freezing is the mode of resistance.

#### DEPRESSION OF FREEZING POINT: OSMOTIC VALUE

The importance of depression of the freezing point in relation to frost resistance lies not so much in the lowering of the temperature at which ice begins to form—because the difference is only a few degrees at most—as in the reduction in the amount of ice formed, or the increase in the amount of water unfrozen at any temperature below the freezing point. A twice-molar concentration of sugar has a freezing point only  $3.25^{\circ}\text{C}$ . lower than a quarter-molar, but at  $-15^{\circ}\text{C}$ . the amount of ice formed is 75 per cent. and 97 per cent. respectively, and the unfrozen water is 25 per cent. and 3 per cent. of the total water. Since at equilibrium “suction tension,” and therefore the freezing point, must be the same in every part of a cell—vacuole, protoplasm, and cell wall—the osmotic value of the cells measured plasmolytically tells us the freezing point of the whole tissue. Experience supports theory in this regard. WIEGAND's observations prove that ice, though its locus is intercellular, only begins to develop at the freezing point of the cell sap and completely melts at the same temperature. In the following discussion, therefore, we shall refer to the osmotic value or osmotic pressure of the cells rather than to the freezing point of the tissue.

In our attempt to find a correlation between hardiness and osmotic pressure (LEVITT and SCARTH, 25), ten species and more varieties were tested. The two tender herbaceous species (sunflower and castor bean) were incapable either of becoming frost resistant or of increasing in osmotic pressure when exposed to “hardening” temperatures. The two semi-hardy herbaceous species (cabbage and clover) showed an increase of 20 to 30 per cent. in osmotic pressure as a result of hardening. The six hardy woody plants possessed maximum winter osmotic pressures up to 400 per cent. greater than their minimum in spring. Among these, the highest concentration was found in the hardest species, *Caragana*, and it also reached its maximum earlier in the season than did the others. Of the four apple varieties, one of the two hardy ones showed the highest winter osmotic pressure, whereas the other was no different from the two more tender varieties. However, both reached their maxima earlier in the season than the tender varieties.

In short, our results reveal wide seasonal changes in the osmotic pressure, and support the general principle of a correlation between osmotic pressure and hardiness; but they also show that the correlation is not close nor invariable. In particular, they indicate that osmotic pressure rises to a high



value in many woody plants before there is actual need of hardening, and may remain constant during the subsequent period of falling temperature. Unfortunately, we have as yet no tests of the actual hardiness of the plants during this period. An example of the yearly cycle, that of *Hydrangea paniculata*, is shown graphically in figure 1.

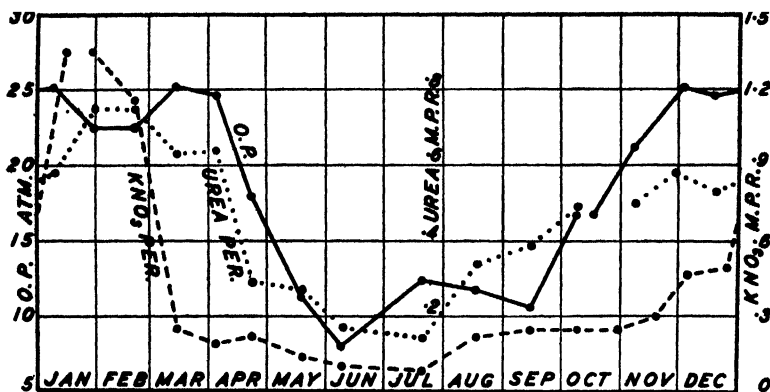


FIG. 1. Seasonal changes in osmotic pressure and urea permeability (Oct. 1934–Oct. 1935) and in KNO<sub>3</sub> permeability (Jan. 1935–Jan. 1936) of cortical cells of *Hydrangea paniculata*.

The depression of the death point, or temperature at which plants are quickly killed, is relatively far greater than the depression of the freezing point of their saps in “natural” hardening, while it is only equal to it in the hardening produced by artificially increasing the sap concentration. ÅKERMAN found that when the osmotic value of the cabbage cells was increased from the equivalent of 0.4 M to 1.0 M sugar, by allowing the leaves to take up erythrol, the death point was lowered from  $-2^{\circ}$  to  $-5^{\circ}$  C. The change in each is 150 per cent., but we find in natural hardening of the same cells that 150 per cent. lowering of the death point is attended by only 20 per cent. increase of osmotic pressure.

#### REDUCTION IN FREE WATER

The smaller the proportion of freezable water in the cell, the smaller the amount of potential ice. Furthermore, if free water be synonymous with solvent water, its reduction will raise the osmotic pressure and reduce freezing in that way. The percentage of free water may be lessened in three ways: (a) loss of water; (b) increase of solids; and (c) binding of water.

Loss of water in hardening is usually slight and has already been discussed. Increase of solids must of necessity depend upon nutrition and storage, and cannot result from temperature effect alone; but it may be of great importance in frost resistance. The binding of water requires some discussion.

One of the commonest statements in recent literature on winter hardiness is that an increase in cold resistance is associated with an increase in hydrophilic colloid, or, in other words, a greater proportion of "bound water." The evidence for this statement in the work of ROSA, GORTNER, NEWTON and others, though based on different methods of estimation, is all open to criticism and is opposed by some recent findings (MEYER, 32; MARTIN, 30; LEBEDINCEV, 23). Some authorities, such as A. V. HILL, even assert that there is no bound (in the sense of non-solvent) water even in animal tissues, where we should expect a higher ratio than in plants. When GROLLMAN'S formula (16), which makes due allowance for hydration of sugar, is used to calculate bound water in wheat juice from GORTNER'S cryoscopic measurements, the percentage of bound water is found to have a quite small and sometimes negative value. The negative value is explained by GORTNER (14) as due to preferential adsorption of solute.

There are several objections to estimation of bound water on plant juices, among them being difficulties of sampling and possible change, with death, in the hydrophilic properties of the colloids.

With living cells it is not possible to measure bound water directly, but, more to the point, free or solvent water can be determined accurately, and from this, if desired, some estimation of bound water may also be reached. As details of methods and results have been given in a preceding paper (LEVITT and SCARTH, 25) a summary will suffice here.

If the cell is simply a pure solution surrounded by an osmotic membrane, then it will obey BOYLE'S law, so that  $P \propto \frac{1}{V}$ . If, however, there is an appreciable quantity of non-soluble solids or of non-solvent (i.e., "bound") water, then this relation will not hold. The formula  $P \propto \frac{1}{V-x}$  must then be used,  $x$  representing that fraction of the cell volume occupied by non-soluble solids and/or non-solvent water.

In determining which of these interpretations of  $x$  is valid, an indirect method must be used. Thus, if  $x$  is constant for all values of  $P$ , then it represents non-soluble solids, since no change in pressure can alter their volume. If, on the other hand,  $x$  varies inversely with  $P$ , then it is at least partially composed of non-solvent water, since bound and free water are in equilibrium.

Thus, an estimation of  $x$  was found to be useful in determining the relationship between bound water and hardiness. In both hardened and unhardened cells of cabbage  $x = 0$  and there is, therefore, no appreciable amount either of non-solvent water or of non-soluble solids in the sap. In cortical cells of *Catalpa* and *Liriodendron*, however,  $x$  is large—about 40 per cent. in hardened and 25 per cent. in dehardened twigs. Since it varies with osmotic pressure,  $x$  must be partially composed of bound water. Figure 2 allows a

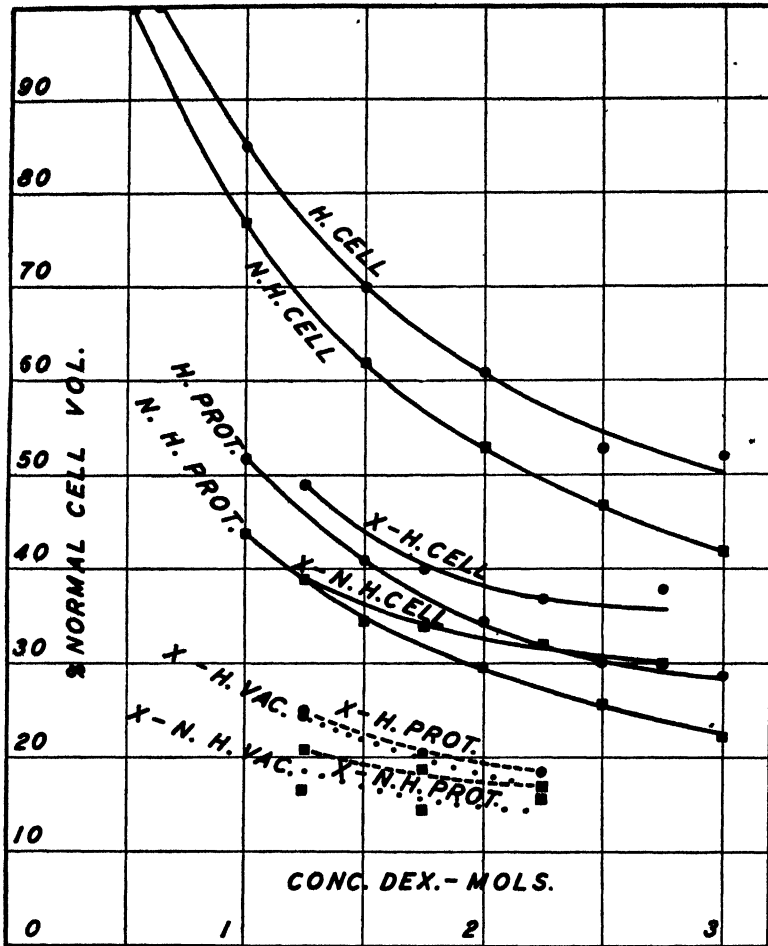


FIG. 2. Change in volume and in non-osmotically active portion ( $x$ ) of *Catalpa* cortical cells (as well as of protoplasm and vacuole separately) with change in concentration of plasmolyte. The volume of the vacuole is shown by the difference between that of the whole cell and of the protoplasm.

comparison to be made between hardened and dehardened cells as regards the cell volume and the proportion of the cell occupied by  $x$  in various concentrations of plasmolyte. The osmotic pressures at normal volume differed only slightly, being 14 atm. in the partially dehardened, and 18 in the hardened; the curves of cell volume tend to diverge in higher concentrations, due to the effect of  $x$ . Greater divergence is shown when dehardening is more complete (25).  $X$  is proportionally larger in the vacuole than in the protoplasm, and it is here that most of the difference in  $x$  between hardened and dehardened cells resides.

Seasonal changes are also significant. Early in May,  $\pi$  drops to half its winter value in both *Catalpa* and *Liriodendron* cells, and since this happens before any appreciable growth, it cannot be due to metabolic utilization of non-soluble solids. Nor can it be caused by a simple hydrolysis of the latter to soluble substances, for in this case a rise in osmotic pressure would result, whereas the reverse occurs. Thus the only possible cause of the seasonal drop in  $\pi$  at this period is a decrease in bound water. A further diminution of  $\pi$  occurs later in the season, but this may be due to the development of new growth.

Briefly then, determinations of the non-osmotically active fraction of the cells of woody plants reveals the existence of bound water which occurs in greater quantity in hardened than in unhardened cells, and which partly accounts for the very high winter osmotic pressures possessed by the former. A semi-hardy herbaceous plant, however, was found to have no measurable amount of bound water either in the hardened or unhardened condition.

The importance of increase of non-solvent space in reducing freezing may be illustrated by a calculation given in a previous paper, which shows that, whereas in non-hardened *Catalpa* 75 per cent. of the cell volume is converted into ice at  $-6^{\circ}\text{C}$ ., in hardened tissue this amount is probably never reached at any temperature. In view also of the fact that  $-6^{\circ}\text{C}$ . is about the critical temperature for unhardened cells (95 per cent. were killed in six hours), the extremely low temperature that the hardened cells endure is not surprising.

Important though this factor may be in extreme resistance, it is not likely to be the only one. Moderate hardening is produced in cabbage without it, and more than can be explained by the osmotic increase. We look, therefore, to other theories of resistance.

## II. Resistance to physico-chemical effects of dehydration

Reduction in the amount of solvent water as a consequence of ice formation results in concentration of the cell sap, which many authors, from GORKE (13) and LINDFORSS (29) onward, have regarded as producing death of the cell by virtue of a toxic effect. More precisely, the mechanism is often pictured as a direct flocculation or even salting out of the protoplasmic colloids by coagulating substances, principally electrolytes present in the sap. There are many theories as to the means by which hardening tends to obviate this result. Protective changes have been detected or assumed, both in the composition of the sap or aqueous phase and in the protoplasmic colloids themselves.

It would be highly desirable to know what happens in the aqueous phase of the protoplasm, as distinct both from the vacuolar sap and from its own colloids, but this is difficult. In dealing with press juices, we may separate

the coagulated colloids from a liquid which, in varying but usually very small part, is derived from the protoplasm but is mainly vacuolar sap. In studying living cells, we may measure properties of vacuole and protoplasm separately but cannot distinguish the phases of the latter.

Since in our own research the approach to the problem is the cytological one, we shall classify the changes according as they appear in the vacuolar sap or in the protoplasm, respectively.

#### CHANGES IN THE CELL SAP

If freezing kills cells through toxic concentration of the sap, resistance to frost injury might be increased, either by reduction in the amount of toxic substances present or by increase in protective substances.

The agents to which the toxic action has been ascribed are electrolytes in general—in virtue of their precipitating action on colloids—and acid in particular. Protective action has been ascribed principally to sugars as inhibitors of protein coagulation. We shall consider these possibilities in turn.

(a) CONCENTRATION OF ELECTROLYTES.—It has been tacitly assumed by most investigators that any increase in the concentration of the cell sap which occurs on hardening is due to an increase solely of organic solutes. Determinations of electrolytes, when these have been made, have generally failed to show any change (DIXON and ATKINS, 9; LEWIS and TUTTLE, 27; NEWTON, 37). Recently, however, DEXTER (5, 7) demonstrated a definite decrease in electrolytes amounting to 50 per cent. (per gm. dry matter), during the hardening period of wheat seedlings, but no change in alfalfa. GREATHOUSE and STUART (15) also have reported a decrease in red clover. We have yet to learn if this is a widespread phenomenon among plants. DEXTER himself is of the opinion that the removal of salts by diffusion or any other process does not afford any protection to the plant or its sap. Our own results show that both hardened and unhardened cells can be made to take up very large amounts of  $\text{KNO}_3$  without suffering injury. Also the evidence of ash content, even for wheat seedlings (NEWTON 37) is against any change in concentration of inorganic salts. The decrease must be in organic electrolytes.

(b) HYDROGEN ION CONCENTRATION.—SCHNADER and SCHAFFNIT (45) put forward the hypothesis, also advocated by HARVEY (17), that on concentration of the cell sap the H-ions first reach toxic limits and that frost injury is really acid injury. In support of this, ZACHAROWA (48) states that the more acid tissues in a plant die first. Most of the facts, however, are opposed to the theory of acid injury.

Thus, hardening is not accompanied by any significant change in the pH of tissue juices, as was proved by ROSA (42), BAKKE *et al.* (2), NEWTON

(36), DEXTER *et al.* (8), and DOYLE and CLINCH (10). Our own results show a very slight tendency to reduction of acidity in the sap—a pH change of at most 0.2—in hardened cabbage seedlings. This was found also by DEXTER (6), KESSLER (22), and GREATHOUSE and STUART (15). In no case except some of KESSLER's results is the change sufficient to offer appreciable protection. Since, however, it is possible that pH differences might exist in the respective cytoplasm, unaccompanied by corresponding differences in the sap, we approached the problem from another angle and tried the effect of changing the pH of the cells.

Following SCARTH's (44) method of altering the pH of living cells, unhardened cabbage seedlings enclosed in bell jars were exposed to the vapor of varying concentrations of ammonia and acetic acid. Solutions of indicators as well as living leaves of *Zebrina pendula* included in the bell jar showed that the series of pH's obtained probably ranged between about 4.4 and 7.5. While still exposed to the vapor, the plants were subjected to frost in a cold chamber. The temperature used (about  $-5^{\circ}$  C.) was sufficient to inflict severe injury on unhardened seedlings, but insufficient to cause much damage to hardened ones. In all cases, both the treated and the control plants suffered between 90 and 100 per cent. injury. KESSLER, allowing plants to take up urea to increase the cellular pH, also found no change in resistance.

Mention may also be made of experiments with expressed juice. First, as found by DEXTER (6) and independently by ourselves (LEVITT, 24), the juice of hardened plants of cabbage is no better buffered and consequently offers no greater opposition to increase of acidity than that of unhardened plants. Secondly, the proteins in the juice of hardened tissue, instead of being more stable toward acid, are more completely precipitated on the acid side of the iso-electric zone than in the unhardened juice. This last point anticipates the discussion of protoplasmic changes, but is mentioned now to complete our argument against the theory of acid injury.

(c) SUGAR CONCENTRATION.—LIDFORSS (28, 29) put forward the theory that the sugars, increase of which is responsible for practically all of the osmotic change in hardening, afford a more important protection than mere osmotic action; namely, a specific protection against coagulation of the protoplasmic colloids, analogous to, but perhaps greater than, the protection which they afford to proteins *in vitro* (NEWTON and BROWN, 38). Whether protoplasm after hardening shows any greater stability, under the action of coagulating agents, will be discussed in the next section. Meanwhile, we may judge the protection theory on the basis of the correlation between sugar concentration and hardness.

Dependence of some plants upon carbohydrate concentration for hardening seems to follow from the fact that, in the seedling stage when there is no

carbohydrate reserve, they are unable to harden properly if kept in the dark (TUMANOV, 46; DEXTER, 4).

On the other hand, as LIDFORSS himself admits, many plants (such as beet and sugar cane) with high sugar content are killed by light frosts, while others (such as mosses and bacteria) with little sugar are highly resistant.

Also, a single species or individual plant may increase the sugar content of its tissue without any increase of hardiness, as shown in the following results.

An experiment of our own with a tender type of plant may be mentioned first. Sunflower plants were grown in the greenhouse for 90 days. The osmotic pressure was low at first (10.6 atm.), later increased (12.4 atm.), and finally, as the flower bud formed, reached its peak (14.3 atm.). Yet neither this increase in osmotic pressure, nor exposure to low temperature, enabled them to assume any frost resistance. The plant is tender at all stages.

Artificial increase of sugar concentration does not produce hardening, even in plants which are capable of it. DEXTER (6) showed that when cut leaves of cabbage were set in sugar solution, the osmotic pressure of their cells increased considerably but their frost resistance was not appreciably altered. KESSLER (22) obtained similar results, using evergreen plants and glycerine solutions.

From the discussion of osmotic pressure in a previous section, it appears that a correlation between sugar concentration and hardiness is common, but we see from the above that plants may be hardy without high sugar content, and also that increase of sugars apart from other change affords no protection beyond its osmotic effect.

#### CHANGES IN THE PROTOPLASM

On the theory that death results from dehydration of the protoplasm, the defense mechanism might be either an increase of resistance to the process of dehydration or a decrease of sensitivity to its effects. We shall call these protoplasmic properties hydrophilily, and stability, respectively.

(a) **HYDROPHILY.**—Protoplasm being surrounded by a semi-permeable membrane, its attraction for water is osmotic, but osmotic pressure in turn may be profoundly affected by binding of water on its colloids. This latter phenomenon has been investigated mainly on extracted juice, involving coagulation of the protoplasm, which may obscure any change in the hydrophilily of the colloids produced by hardening. Even negative results, therefore, would not disprove the hypothesis that hardening is associated with greater hydrophilily of the protoplasm. The question can be settled only by a study of living cells, and we have attempted this in two ways: (1) comparing the average volume occupied by the protoplasm in hard-

ened and unhardened tissue; and (2) by estimating the bound water from variation of non-solvent space in relation to degree of plasmolysis.

(1) *Comparison of the average volume occupied by the protoplasm in hardened and unhardened tissue.*—Since the total pressure of the protoplasm, in whatever ratio it is divided between swelling pressure and osmotic pressure, must equal the total pressure in the vacuole, an increase in the hydrophily of the protoplasm must result in transfer of water from the vacuole into it until equilibrium is again established. An increase in the volume of the cytoplasmic layer would be the visible result. Of course, changes in the osmotic pressure of the sap must be taken into account, but, allowing for this, measurement of the thickness of the cytoplasm before and after hardening should help to settle the problem of whether its hydrophily changes or not.

We first attempted to make the measurements of the protoplasmic caps in epidermal cells of bulb scales of *Allium cepa* which does not form starch. The result, if there were no complications, pointed to a *decrease of hydrophily in the protoplasm on hardening* (tables I and II).

TABLE I

TOTAL CROSS-SECTIONAL AREA (SQ.  $\mu$ ) OF BOTH PROTOPLASM CAPS (AVERAGE OF 10 CELLS)  
FROM PLANIMETER MEASUREMENTS OF CAMERA LUCIDA DRAWINGS

ONION NO.	DAYS AT 25° C. (PREVIOUS TO HARDENING)	UNHARDENED	HARDENED	CHANGE
1 .....	7	300	306	+ 6
2 .....	"	303	281	- 22
3 .....	14	376	338	- 38
4 .....	"	477	370	- 107
5 .....	28	376	314	- 62
				Average, - 45

TABLE II

COMPARISON OF 10-CELL SAMPLES

ONION NO.	SAMPLE a	SAMPLE b	DIVERGENCE
1 .....	309	319	10
2 .....	370	389	19
3 .....	284	296	12
			Average, 14



The volume of such caps, however, is affected by other factors than volume of the whole cytoplasm—factors such as surface tension of the vacuole, and vacuolar contraction. The result is therefore indecisive.

More satisfactory determinations of volume were made on the cortical cells of apple twigs. These cells possess very bulky protoplasm, occupying about half the cross-sectional area of each cell. They contain no starch in the hardened state, but an abundance of it when in the naturally unhardened condition. Artificial dehardening of branches taken into the laboratory, however, is accomplished before any starch appears, and it was during a two-week dehardening period of this kind that the measurements were made. The average of four branches shows a practically constant volume of protoplasm during this period of dehardening (table III). Since osmotic pressure fell during the same time from 25.5 to 19 atm.—about 25 per cent. reduction—the pressure in the protoplasm must have fallen *pari passu*, indicating, if due to colloidal change, a slight decrease of hydrophily, or conversely a *slight increase of hydrophily (though not of hydration) with hardening*. On the other hand, the change may be purely osmotic.

TABLE III

TWIGS TAKEN INDOORS FEBRUARY 11, 1935. EACH VALUE IS THE AVERAGE OF 10 CELLS. AREAS OBTAINED FROM PLANIMETER MEASUREMENTS OF CAMERA-LUCIDA DRAWINGS

TIME IN LABORATORY	PERCENTAGE OF CELL AREA OCCUPIED BY PROTOPLASM				
	ALEXANDER	WEALTHY	WOLF RIVER	PATTEN GREENING	AVERAGE
<i>days</i>	%	%	%	%	%
0 .....	47.7	45.2	53.1	47.7	48.4
1 .....	46.3	42.2	53.0	57.4	49.7
3 .....	50.5	46.3	49.5	51.7	49.5
7 .....	52.6	51.1	54.4	49.6	51.6
14 .....	46.7	41.3	50.8	57.6	49.1

Still another comparison was made, using the cortical cells of *Catalpa*. In this case the proportion of protoplasm was found to decrease definitely on dehardening, but the beginnings of growth introduced a complicating factor (fig. 2).

While these results seem to preclude any very great or general increase in protoplasmic volume with hardening, such as the theory of protoplasmic resistance to dehydration would demand, they do not reveal how much of the increase of osmotic value which does occur is due to colloidal change. The next method gives more definite results on this problem.

(2) *Non-solvent space and bound water in the protoplasm.*—It has already been shown that the non-solvent space in hardened cortex cells is

larger than in unhardened cells, and that most of the increase occurs in the vacuole. The protoplasm also possesses a large proportion of non-solvent space—about 50 per cent. of its volume when in equilibrium with a molar dextrose solution, both in hardened and dehardened cells. This is about the same proportion as in the vacuole of dehardened cells, but only two-thirds of that in the vacuole of hardened cells.

Since in the results with *Catalpa*, as shown in figure 2, the volume, both of the protoplasm as a whole and of its non-solvent fraction, is greater in the hardened cells, it follows that the protoplasmic colloids are more hydrophilic.

But even the absence of an increase in the volume of the protoplasm with hardening would not preclude a possible increase of hydration or hydrophilic quality, because of the complication that the dry weight of the protoplasm may diminish. In absence of photosynthesis, there must be a reserve of insoluble material in the cell, which, during the hardening process, is transformed into osmotically active substance and, since simultaneously with the osmotic increase the non-solvent space in the vacuole increases greatly while in the protoplasm it shows an increase only when calculated in relation to the normal cell volume, the diminution of solids is more likely to be in the latter. Commonly, starch is the visible substrate, and that of course is stored in the plastids; but in the starch-free cells, on which our experiments were mostly made, some intermediate reserve carbohydrate or fat may be present in a state of dispersion in the cytoplasm also.

If indeed the protoplasm loses solids to the vacuole, and since its own non-solvent space is undiminished or even increased, the bound water element in it must be augmented. In other words, its colloids become more hydrophilic.

On the same condition, since the volume of the whole protoplasm remains the same, its total water content must be higher.

These volume studies are beset with difficulties and cannot be regarded as more than preliminary, but at any rate on the whole they support the theory of an increase in hydrophilicity of the protoplasm with hardening.

(b) STABILITY.—This property is closely related to the previous one, since the resistance of the protoplasm, or any other colloidal system, to precipitation or salting out, depends largely on the hydrophilicity of its least stable elements. But an increase in the hydrophilic property of a portion of the solid phase of the protoplasm would not be reflected in a proportional increase in that of the protoplasm as a whole; so that a great increase in protoplasmic stability may attend only a slight increase in its attraction for water, as estimated by the methods described in the previous section. Also, there are probably factors other than hydrophilicity concerned in such a complex phenomenon as coagulation of protoplasm.

As a possible clue to the relative stability of protoplasm in the hardened and unhardened state, respectively, we may compare its resistance to other coagulation agents than frost. The action of acid and of heat was tried. Hardened and unhardened cabbage plants were exposed together to vapor of a 7.5 per cent. and also of a 10 per cent. solution of acetic acid for 5 hours. All of the plants suffered, but parts of them remained alive. Hardened and unhardened suffered alike.

The heat test was made by dipping leaves into water at 60° C. long enough to produce partial killing. Again the result was the same in hardened and unhardened plants. *There is no indication here, therefore, of protoplasm becoming less sensitive to coagulating agents in general as a result of hardening.*

HARVEY, who is the author of the theory of greater protein stability, bases his hypothesis on tests with expressed juice. He found (17) that when the juice of hardened and unhardened cabbage leaves was frozen and, centrifuged, chemical analysis revealed that the precipitation of proteins was greater in the latter. MUDRA (34) obtained the same result with other plants. HARVEY also found a greater percentage of soluble amino acids in hardened juice, though NEWTON *et al.* (39) regard this as an effect of freezing rather than of hardening.

Testing cabbage juice in relation to the H-ion effect, we obtain the contrary result as regards stability. To a series of tubes each containing 10 cc. of tissue extract, various concentrations of 0.1 N HCl were added. The region of complete precipitation was from pH 3.9 to pH 4.4 in the case of both hardened and unhardened plants. On either side of this zone is a region of incomplete precipitation, the limits of which are pH 5.2 and pH 3.9 in the unhardened, whereas in the hardened plants they extend to pH 5.4 and pH 3.5. Beyond this zone there is little or no precipitation on either acid or alkaline side. *The wider pH zone of precipitation in the juice of hardened plants would point to a poorer stability of their colloids.* This experiment goes to offset results from which a more hydrophilic quality of the juice colloids has been inferred. But of course juice is not protoplasm!

Changes in the colloidal stability of a hydrophilic sol are often paralleled by changes in viscosity, since viscosity is directly and profoundly influenced by the hydration of the internal phase. But the so-called viscosity of protoplasm, as it is measured, is rarely true viscosity, and is influenced by aggregation as well as hydration of particles. Viscosity tests and their significance are reserved for a later section of this paper.

Certain facts are at variance with any theory of injury through dehydration; for example, the experience that drought-resistant plants are not always frost-resistant, and the finding of ILJIN that cells of plants which are sensitive both to light frost and to wilting may survive extreme and pro-

longed desiccation, when this is produced by plasmolysis alone or plasmolysis followed by drying. These phenomena point to mechanical factors as the immediate cause of death, both in freezing and wilting, though not exactly the same in the two cases. Moreover, the most conspicuous of the changes so far described—those producing reduction in ice formation—are as applicable to a theory of mechanical as to one of dehydration injury.

### III. Resistance to mechanical effects of freezing and thawing

Theories of mechanical injury may be subdivided into two sets: one which ascribes the effect to pressure of ice crystals, and the other to stress set up by displacement of water. In the former case the fatal period will be that of freezing; in the latter it may be either freezing or thawing. An advantage in the study of mechanical as compared with physico-chemical action is that it can be followed microscopically.

#### INJURY THROUGH PRESSURE OF ICE

The view that frost injury is mechanical dates far back. DUHAMEL and BUFFON (11) postulated that ice forming inside a cell ruptured the wall by expansion. From direct observation, however, it was proved by GÖPPERT (12) that the walls are not ruptured, and by SACHS (43), MOLISCH (33) and others that normally ice forms in the intercellular spaces. Later it was shown that with rapid freezing intracellular development of ice does occur (MÜLLER-THURGAU, 35; MOLISCH, 33). This condition is generally fatal to plant cells (ÅKERMAN, 1; CHAMBERS and HALE, 3), whereas some animal tissues are said to be less injured by quick than by slow freezing, because the smaller crystals produce less destruction of the tissue. In plant cells, if the vacuole freezes, the compression of the protoplasm between it and the wall, especially if there is a hull of ice outside (ILJIN, 19), is probably sufficient to cause death, even if the protoplasm itself resists freezing. But opinions differ as to the exertion of mechanical pressure by ordinary intercellular ice. MAXIMOV is a leading upholder of the theory that pressure of the ice crystals on the cells induces coagulation of the protoplasm, and he couples with it the suggestion that mere contact of the ice phase with the plasma membrane may be fatal. On the other hand, SCHANDER and SCHAFFNITZ (45), followed by ILJIN, argue that the ice crystals grow into the intercellular spaces and neither press upon the cells nor even touch the protoplasm. ILJIN points out that copious intercellular space in a plant is no protection against frost. Yet it has long been known that cells and tissues may be torn apart by the growth of relatively large ice masses (PFEFFER, 41), and WIEGAND's observations of frozen buds and twigs indicate that less obvious pressure is exerted by crystals of smaller and more ordinary dimensions. There is lack of proof, however, that this is a general cause of injury.

The phenomenon with MAXIMOV first quoted in support of the theory, *viz.*, the protective action of external solutions (which reduced the amount of ice), was shown by ÅKERMAN (1) and ILJIN (19) to depend mainly on plasmolyzing action, and thus pointed the way to an alternative type of mechanical theory.

#### INJURY THROUGH STRESS SET UP BY DISPLACEMENT OF WATER

WIEGAND (47) and others observed that when tissues freeze in the ordinary way the cells shrink, wall and all, as water is withdrawn. HOLLE (18) noted the same thing in wilting. This behavior is made possible by liquid cohesion within the cell, and adhesion of protoplasm to wall, there being no external liquid to cause plasmolysis. In proportion to the rigidity of the wall, stresses are set up in the cell by its loss of water, and ILJIN observed that in drying cells the protoplasm might be ruptured within itself or torn from the wall—with fatal effect. This he at first regarded as the regular cause of injury, whether in frost or drought, because, when the protoplast was released from the wall by true plasmolysis, the cells could endure severe desiccation and freezing. Later experiments (20) led to a modification of his view, with regard to frost injury at least. He found that application of solutions *after* freezing was also an effective protection, and therefore he assigned injury to the period of thawing. What happens in thawing, according to his observations, is that the walls first absorb water and lift away from the protoplast (pseudoplasmolysis), after which deplasmolysis tends to ensue. He avers that, on account of its high viscosity in the dehydrated state, the protoplast ruptures if deplasmolysis is rapid. Accordingly he argues that the protective function of the solutions bathing the cell is to slow down deplasmolysis. Whether ILJIN's theory is correct in detail or not, it draws attention to a type of stress to which cells are exposed, and leads us to inquire what kind of changes would enable the cells to avoid mechanical injury under these circumstances.

#### HARDENING CHANGES IN RELATION TO MECHANICAL INJURY

As already mentioned, some of the changes discussed under dehydration injury are just as applicable here. Part of the osmotic increase is almost always due to lowering of moisture content which, while it reduces the amount of ice and of mechanical injury, is itself a stage in dehydration and not a protection against this result. (*Cf.*, however, its possible rôle in supercooling, as described previously.) The binding of water, while actually a disadvantage as regards undue concentration of the sap, may be very useful against mechanical injury.

The supposed protective action of sugars has also been invoked by MAXIMOV in the case of protoplasmic coagulation from mechanical causes.

But there are certain other properties of the protoplasm which must play a part in resistance to mechanical injury such as we have described, and which have been neglected almost entirely in studies of the mechanism of frost resistance. Such are (a) the permeability and (b) the viscosity of the protoplasm.

(a) PERMEABILITY.—In a previous report (LEVITT and SCARTH, 26) we have demonstrated that the permeability of cells for polar compounds increases remarkably with hardening and that it undergoes a greater change and is better correlated with hardness than any other character, even osmotic pressure. Towards apolar non-electrolytes, such as urethane and succinimide, there is no measurable change. Towards polar non-electrolytes, such as urea, thiourea, and glycol, which have relatively small molecules, there is a general relation between permeability and hardening, but the seasonal changes do not always run parallel. Towards an electrolyte ( $\text{KNO}_3$ ) the variation is still greater and the correlation with hardness much closer. Data regarding water permeability are insufficient to allow of an equally general statement, but as far as they go they show the same trend as with other polar compounds. Cabbage cells are about twice as permeable to water when hardened as when not, and those of woody plants show a greater difference. On the membrane theory of permeability we draw the general inference that there is a widening of the aqueous pores of one or both the protoplasmic membranes of the cell.

Our results show that the correlation between permeability and hardness exists in various types of plants and shows no exceptions. Also the relation is independent of the nature of the cause which induced the hardening. Low temperature, drought, checking of growth, all increase permeability as well as hardness.

To some extent the relation seems to hold even in the vegetative, unhardened phase of life. Thus, highly cold resistant cells, such as those of bacteria, mosses, and cortex of woody plants, are at all times and seasons unusually permeable to  $\text{KNO}_3$  and perhaps to water.

In view of this widespread association of the two conditions and of the fact that a permeability change does not necessarily attend an osmotic change and is therefore not simply incidental to the latter, it seems likely that there is a direct causal connection between permeability and hardness. This will be discussed later.

(b) VISCOSITY OF PROTOPLASM.—If, as ILJIN finds, injury may occur through tearing of the protoplast in a phase of thawing out, we might expect to find protection through an increase of plasticity or reduction of viscosity in hardened cells. A comparison of the viscosity of protoplasm in the hardened and unhardened state has been made by KESSLER (22), who arrived at the conclusion that viscosity increased, not decreased, in hardening. He

tested the cells of various plants in summer and winter by the centrifuge as well as by the plasmolytic method. The centrifuge method in this case is handicapped by the fact that starch is present in the unhardened and absent in the hardened condition of plants. From subsidiary experiments with darkened plants, KESSLER reached the astonishing conclusion that the specific gravity of starch-free plastids is higher than of those with starch—in spite of the fact that starch is much denser than protoplasm in general, while chloroplasts are believed to be rather lipoidal in composition, and were actually found by himself to have a tendency to move *centripetally* in some hardened tissue. If KESSLER's premise as to specific gravity is at fault, his result that chloroplasts in unhardened plants are more easily thrown down than in hardened is no proof of lower viscosity.

The "plasmolysis time" test was applicable only to *Sempervivum* among the plants tested by KESSLER, where it gave a comparable result. Slower rounding up in the hardened cells pointed to a greater viscosity—or rather a stronger adhesion to the cell wall—in the hardened state. The other genera used, *Hedera* and *Saxifraga*, did not allow comparison, because the protoplast rounded up immediately on plasmolysis.

In our own work on cold resistance, the attempt was first made to distinguish a viscosity difference by observing Brownian movement. However, neither streaming nor any appreciable Brownian movement could be detected in hardened *Catalpa* cells. Nevertheless, the plastids and protoplasm seemed to be clumped together at the periphery and mostly the ends of the cells, perhaps indicating a low viscosity which would allow free play to surface tension. In the dehardened cells, on the other hand, streaming was active and Brownian movement was apparent in the currents. Yet, if the dehardened cells were observed immediately after sectioning, no streaming and little or no Brownian movement was discernible. The protoplasm showed much less peripheral clumping than in the hardened cells. Hardened and unhardened cabbage cells both exhibited Brownian movement, but no difference in the activity could be distinguished.

Plasmolysis shape was next observed. Sections of hardened and dehardened *Catalpa* were placed on a slide in an isotonic solution of  $\text{CaCl}_2$  (0.30 M and 0.18 M, respectively) which was allowed to evaporate in the air. At the end of four hours the cells were strongly plasmolyzed in both, but the shape differed. The hardened cells were well-rounded, the dehardened ones were strongly concave, adhering to the wall in many places (fig. 3).

In the case of cabbage, sections from hardened and unhardened seedlings were placed in twice isotonic dextrose and examined from time to time. Though the difference was not so striking as in the case of *Catalpa* cells, the rate of rounding up appeared somewhat more rapid in the hardened cells (table IV).

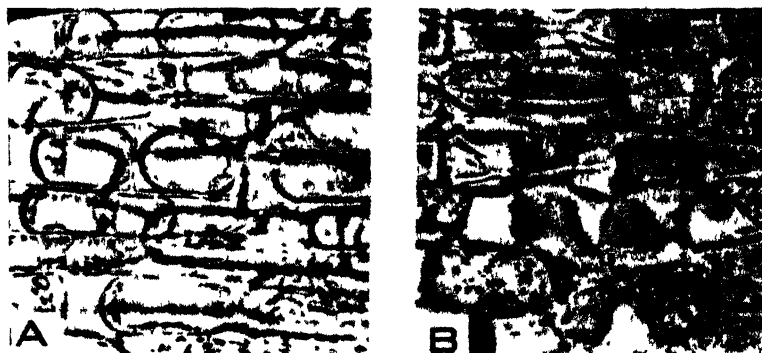


FIG. 3. Plasmolysis shape in hardened (A) and dehardened (B) *Catalpa* cells in isotonic  $\text{CaCl}_2$  solutions after evaporation in air for six hours.

TABLE IV

RATE OF ROUNDING UP ("PLASMOLYSIS TIME") OF UNHARDENED AND HARDENED CABBAGE CELLS IN TWICE ISOTONIC DEXTROSE. EACH AN AVERAGE OF THREE PLANTS

SAMPLES	NON-HARDENED		HARDENED	
	OSMOTIC PRESSURE	PLASMOLYSIS TIME	OSMOTIC PRESSURE	PLASMOLYSIS TIME
1.	10.0	90	13.6	60
2.	10.6	75	13.6	60
Av.	10.3	82	13.6	60

RESISTANCE TO DEPLASMOLYSIS.—Another mode of experimentation also gave definite and positive results. Sections of hardened and unhardened cabbage plants were compared as regards the ability of the protoplasts to withstand the stretching caused by rapid deplasmolysis. The tendency to injury from this cause generally increases with viscosity of the protoplasm.

First the cells were plasmolyzed for 15 to 20 minutes in twice isotonic  $\text{CaCl}_2$  and then transferred to distilled water. To determine the number of cells surviving deplasmolysis, the sections were once more transferred to the plasmolyzing solution. The results are presented in table V.

Tender plants always showed sensitivity to deplasmolysis. Thus, *Cordyline* petiole cells almost all burst when transferred to distilled water from twice isotonic  $\text{CaCl}_2$ . In the case of others (tomato, bean), it was found impossible to determine urea permeability, since even this slow deplasmolysis proved fatal.

Hardened and dehardened *Catalpa* cells were then tested, but in their case more severe treatment was necessary to cause injury. Treatments and results are given in table VI.



TABLE V

COMPARISON OF DEPLASMOLYSIS INJURY IN HARDENED AND UNHARDENED CABBAGE CELLS.  
PLASMOLYZED IN TWICE ISOTONIC  $\text{CaCl}_2$ ; DEPLASMOLYZED IN DISTILLED WATER

CONDITION OF PLANT	OSMOTIC PRESSURE (M $\text{CaCl}_2$ )	PERCENTAGE OF SURVIVING CELLS	
		IN EPIDERMIS AND CHLORENCYMA	IN PITH
Non-hardened	0.16	trace	0
5-day hardened	0.23	most	0
Non-hardened	0.17	few	0
10-day hardened	0.25	all	many

TABLE VI

COMPARISON OF DEPLASMOLYSIS INJURY IN HARDENED AND DEHARDENED *CATALPA* CELLS

CONDITION OF PLANT	TIME IN PLASMOLYTE (MIN.)	NUMBER OF SURVIVING CELLS
	(a) PLASMOLYZED IN 9 PARTS 2M $\text{NaCl}$ : 1 PART 2M $\text{CaCl}_2$ . DEPLASMOLYZED IN DISTILLED WATER	
Dehardened	60	few
Hardened	360	all
	(b) PLASMOLYZED IN 3M $\text{NaCl}$ . DEPLASMOLYZED IN DISTILLED WATER	
Dehardened	15	none
Hardened	15	almost all

Further evidence was obtained indicating that this difference in ability to withstand deplasmolysis injury is even more marked at low temperatures. Thus a much less severe treatment than the above, namely a transfer from twice isotonic to half isotonic dextrose, caused no injury to either at room temperature. Even at  $0^\circ \text{C}$ ., this procedure had no harmful effect on hardened cells but was fatal to the unhardened, due to the increased viscosity in the latter, which was apparently such as to more than counteract the decreased protoplasmal stress resulting from the reduced deplasmolysis rate.

It is thus evident that hardened cells are more resistant to the ill effects of deplasmolysis than are unhardened cells, and this in spite of the more rapid rate in the former.

### Discussion

Reviewing the changes said to be associated with hardening, we have seen that some are not well established and that no single one is adequate to explain the whole phenomenon. Our own research on living cells confirms the importance of certain changes in the sap and brings to light others in the protoplasm. The significance and relative importance of these is difficult

to evaluate from lack of knowledge of the exact mode or modes of injury. The nature of the protection is inferred mainly from the nature of the hardening change.

A wide range of protection is possible with those changes that reduce freezing. Of these, supercooling is not fundamental because hardy tissues survive severe freezing; theoretically, supercooling may even be harmful by leading to intracellular ice development during the quick freezing which follows its breakdown. A rise in osmotic pressure is generally well correlated with hardiness, but in all true hardening it fails to explain more than a fraction of the increased resistance.

Enhancing the osmotic effect in woody plants is a marked increase in "non-solvent space" in the sap vacuole. Part of this is due to insoluble solid—apparently hydrophilic colloid—and part to bound water. This change causes the osmotic pressure to mount more rapidly as the cell contracts and sets a higher limit to the minimum volume which can be reached through water loss. It is calculated that hardened *Catalpa* cells lose no more water through freezing at the lowest possible temperature than the unhardened cells do at only  $-6^{\circ}\text{C}$ .

The gelatinous condition of the sap in tree cells may serve another purpose. In ordinary herbaceous plants, intracellular freezing—when it occurs—is located in the sap vacuole. In other words, this is the vulnerable part of the cell as regards the invasion of ice, and such invasion is usually fatal. But in hardy tree cells the vacuole is as rich in hydrophilic colloid as the protoplasm and probably equally protected from freezing even when the eutectic point of the sap is reached.

Important though these factors may be in resistance to extreme cold, they are not the full explanation of hardiness. For example, in the non-hardy state, tree cells still have much colloid in their sap, but they are then more sensitive to frost than hardened cabbage cells which have no colloid and about the same osmotic pressure.

Other changes in the sap have not been shown to be of any great significance. Of those which are supposed to offset the danger of salting out through concentration of the sap, a reduction, with hardening, of the total electrolytes has so far been proved in one or two cases only and disproved in others. Also, in spite of a permeability increase on hardening, there is no evidence of exosmosis from the cells in winter time. Moreover, a high concentration of salt ( $\text{KNO}_3$ ) has been produced inside cells without injury. As regards the theory of toxic acidity of the sap, a reduction of H-ion concentration in hardening has been found in several cases, but only to a slight degree, while artificial alteration of the acidity of the sap is without effect on frost resistance.

The view that the sugars, which are responsible for most of the osmotic change, also exert a specific protection against coagulation of the protoplasm

is countered by the fact that increase of sugar may take place naturally or be produced artificially without a true hardening effect.

As regards changes in the protoplasm, a widely held view is that hardening is accompanied by an *increase of hydrophilic colloid* and consequently of resistance to freezing, to dehydration, and to coagulation.

In attempting to investigate this problem with living cells, in which alone the hydrophilic property of protoplasm is normal, we have seen that in cases where the protoplasm does not swell appreciably in hardening, it may nevertheless be more hydrated if, as seems probable, its insoluble solids diminish. Where swelling does occur the conclusion is more definite.

Evidence regarding changes in the *stability* of the protoplasmic colloids is not decisive. Contradictory results have been obtained with press juices, and with living cells we find that hardening confers no increase of resistance to the action of acid or heat, agents which coagulate proteins.

Altogether, the possibility that frost injury is a physico-chemical effect of dehydration is opposed by the fact that tender cells may often be deprived of water by other means than frost without ill effect. The alternative is mechanical injury. Whatever the precise nature of this injury, the sap changes which tend to reduce the amount of freezing ought to afford some protection; but as we have seen these are not enough.

It would seem that in addition the protoplasm must become more resistant to the mechanical action. Apart from the evidence of a possible colloidal change, which we have just discussed, proof of two very pronounced protoplasmic changes has been deduced in the course of our work, *viz.*, an increase in permeability and what may for convenience be termed a fall of viscosity. Let us see if these two hardening changes can be fitted into a scheme of protection against such types of mechanical injury as are known to occur. At least three modes of mechanical injury have been recorded by various authors from direct observation of cells.

**INTRACELLULAR FREEZING.**—Ice formation within the cell has sometimes been noted and though confined to the vacuole the result is nearly always fatal. The mechanism of injury in this case may be, as ILJIN suggests, compression of the protoplasm between the freezing and expanding sap on the inside and the cell wall or a rigid hull of ice on the outside, or it may also be laceration of the vacuolar membrane and other structures by the ice crystals.

At any rate, the condition for internal freezing is that the temperature of the cell sap fall below its freezing point. Ice first starts to form on the cell walls outside the cells, where it normally grows at the expense of water which diffuses from cells. If this keeps pace with the fall of temperature, the resulting increase in its concentration will prevent the sap from freezing, but with a sudden drop of temperature or sudden crystallization as a result of supercooling, the rate at which water can pass out of the cell may be the

limiting factor in deciding whether or not ice will penetrate. Here, then, is a condition when high water permeability may mean safety to the cell.

INTERCELLULAR ICE MASSES.—It is frequently found that when plants which are not hardy are exposed to temperatures slightly below freezing point, large ice masses develop locally in the tissues. These in their growth crush the cells in the neighborhood and may even tear the tissues apart. Since the size of crystals is a function of rate of crystallization, macrocrystalline ice is naturally found only when very gradual freezing takes place, but the more moderate aggregates which develop under other conditions may also caused injury to the cells in contact with them. Conceivably, the rate of crystallization may sometimes depend upon the rate at which water can pass out of the cells, so that the higher the cell permeability to water the smaller the crystals and the less the danger of injury from pressure. It must be admitted, however, that the rate of exosmosis of water from even non-hardy cells is such that this hypothesis of protection is less plausible than that applied to internal freezing.

THAWING.—ILJIN, backed by his experiments, has revived SACHS's hypothesis of death during thawing. His results cannot controvert the finding of many authors that death may occur in freezing, but they do seem to prove that with the use of salt or sugar solutions, frozen tissues which are still alive may be saved from dying at the stage of thawing out.

Danger would seem to attend both phases of thawing, namely, pseudoplasmosis, and deplasmolysis. During the former, as ILJIN showed, the water released by thawing is taken up by the cell wall faster than by the protoplast. The former extends quickly to its normal size, while the latter remains contracted for a time. As in true plasmolysis, injury is liable to occur here under certain conditions. If the protoplast adheres to the wall in places, its plasma membrane tends to become disorganized through stretching, or if the external membrane allows water to pass more freely than the vacuolar membrane, the cytoplasm swells and becomes vacuolated. These mishaps in thawing would be avoided if the permeability of the protoplast to water approached that of the wall, and if the permeability of the tonoplast also equalled that of the ectoplast. In other words, increased permeability of the protoplasm, and especially of the tonoplast layer, which normally seems to be the less permeable (HÖFLER), would tend to protect the cell from mechanical injury of this kind. Wall and protoplast would extend together without any pseudoplasmosis.

According to ILJIN's later observations, however, the main damage is caused by the rapid stretching in deplasmolysis. The rate of expansion would only be exaggerated by greater permeability to water, but apparently a compensating change in the physical properties of the protoplasm is another feature of the hardening process.

The cells become much more resistant to injury by rapid deplasmolysis

after true plasmolysis, and we may assume that the same would be the case after pseudoplasmolysis in thawing.

The difference between hardened and unhardened cells in this respect is greater at the low temperature at which thawing out occurs than at room temperature. It is probably the result of a lower viscosity of the protoplasm in hardened plants and also a smaller effect of temperature upon its viscosity.

PLASMOLYSIS DUE TO FREEZING.—That cells do plasmolyze sometimes on freezing with formation of ice between the wall and the protoplast is a matter of observation (*e.g.*, CHAMBERS and HALL, 3), but unlike the previous conditions cited, the injury here is hypothetical. Injury in ordinary plasmolysis is common when the protoplasm is highly viscid and adhering to the wall. Low temperature tends to make protoplasm more viscous and hence more liable to injury of this type. As we have seen, the behavior of hardened cells on plasmolysis is exactly such as to reduce this danger. Another advantage of smoother plasmolysis is that strands of protoplasm are less liable to be pinched off by the investing ice.

COLLOIDAL CHANGES IN HARDENING.—The different behavior of hardened cells, both in deplasmolysis and plasmolysis, is such as may be explained by a lowering of viscosity, either of the protoplasm as a whole or at least of its superficial layer. In a visco-elastic material like protoplasm, such a change in consistency is due to gel  $\rightarrow$  sol transformation rather than the true viscosity reduction of a Newtonian liquid. Increased hydration of particles which should increase *true* viscosity may tend towards solation and reduction of *apparent* viscosity. If this should be accompanied by increased hydration of the protoplasm *en masse*, the effect is still more likely to follow.

These are the colloidal changes which our volumetric experiments and other observations have led us to regard as probable. They are also the changes which increase of permeability points to—as regards the plasma membranes at least.

The chemical nature of the colloids involved does not enter into our work, but inasmuch as the osmotic changes are largely produced by carbohydrate transformation, it is likely that the hydrophilic colloid which occupies so much of the sap of hardy tree cells belongs to the same category. This would explain the simultaneous fluctuation of osmotic and colloid substance. The parallel variation of protoplasmic viscosity, and especially permeability, suggests that proteins and lipoids undergo the same type of change as the carbohydrates.

The linked series of changes associated with hardness as we have described them may be summarized as follows:

1. Complicated hydrolytic breakdown of carbohydrates increases the osmotic pressure of the cell and also in the hardier plants the non-solvent space in the vacuole at the expense of starch and perhaps of other reserves held in the cytoplasm.

2. Due to similar changes in the protoplasmic colloids, the whole cytoplasm, probably, and the plasmic membranes, almost certainly, become more hydrated.

3. As a consequence of this change, the viscosity of the protoplasm is lowered.

4. Because of the change in the membranes in particular, cell permeability is increased.

If all of these changes are causally connected one with another, the correlation of each and all of them with frost resistance would be found, though only one might play a part in it. We have theorized as to the possible rôle of each, but a satisfactory demonstration requires more knowledge of the mechanisms both of injury and of resistance than we yet possess.

### Summary

1. Cell changes in hardening are reviewed and theories of the mechanism of frost resistance are discussed. The following are features of hardened as compared with unhardened cells, according to our own results with the plants named:

Resistance to injury by deplasmolysis is greater.

Rate of rounding up in plasmolysis is greater (*Catalpa*, cabbage, etc.).

(Two differences which may point to lower "viscosity.")

The relative volume of protoplasm and vacuole changes but little if at all (apple cortex, onion).

Resistance to acid and to heat does not change (cabbage).

2. Also the following are features which have been reported in a previous paper:

The osmotic pressure is increased, especially in the hardier woody plants (many woody and herbaceous species).

Non-solvent space (= colloids and bound water), if present in measurable amount, increases markedly (*Catalpa* and *Liriodendron*), but the increase is greater in the vacuole than in the protoplasm (*Catalpa*).

Non-solvent space is practically absent in cabbage, hardened as well as unhardened.

Permeability to polar compounds increases, and the more the increase the hardier the plant (many woody and herbaceous species).

3. The press juice of hardened cabbage plants shows:

Precipitation of colloids over a wider zone in the pH scale.

H-ion concentration slightly lower.

Buffering capacity unchanged.

4. Artificial change in the H-ion concentration of the sap in life does not affect hardness (cabbage).

5. The most pronounced and unmistakable changes in the protoplasm are increased permeability and lowered viscosity; in the vacuole, increased osmotic pressure and (in trees) non-solvent space. Ways are suggested in which all of these protect the cells against mechanical injury due to frost.

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## EFFECT OF LIGHT ON SOLANINE SYNTHESIS IN THE POTATO TUBER

HERBERT W. CONNER  
(WITH SIX FIGURES)

In 1820 DESFOSSÉS (15) discovered solanine, a basic substance in juice expressed from the berries of *Solanum nigrum*. He found (14) it almost insoluble in all solvents tried, except alcohol and acids. Six years later BAUP reported (5) it also to be present in the potato (*Solanum tuberosum*), but he found much more in the sprouts than in the tubers.

The chemical nature of the substance is even yet only partially known. ZWENGER and KIND (51) found that it is stable when boiled with potassium hydroxide, and that it cannot reduce Fehling's solution, but (50) that it can be hydrolyzed by strong acids, giving rise to salts of an alkaloid (solanidine) and a solution which is able to reduce Fehling's solution. Identification of the hydrolytic products has been a gradual achievement of several investigators. It appears (24) that a molecule of solanine is composed of one molecule each of solanidine, glucose, galactose, and rhamnose; and that three molecules of water are split out in the formation of the glucoside whose constituent groups are linked in the order in which they have been mentioned (35, 49). The empirical formula of solanidine is still a matter of disagreement (6, 35, 42, 46, 49), but it is known to be a tertiary base with a secondary alcohol group (42, 46).

The glucoside is interesting for several reasons:

1. It is the only basic-reacting glucoside known (31).<sup>1</sup>
2. It has the foam-producing and haemolytic properties of saponins, yet contains nitrogen.
3. It frequently occurs in poisonous concentrations in market potatoes.
4. It is claimed that lesser concentrations increase intestinal absorption (28) by precipitating sterols (?).
5. It is uniquely toxic to conidiospores of *Cladosporium fulvum* which have been raised on glucose agar (1, 41) although spores taken directly from infected tomato leaves tolerate it better.
6. It contains two sugar groups that have not been found free in the plant. If the availability of rhamnose or galactose, or both, limits the elaboration of solanine then a study of the factors which increase the amount of glucoside formed may indirectly contribute to an understanding of how monosaccharides, other than glucose, arise.

<sup>1</sup> Since neither solanine, nor any glucoside of solanthrene has yet been isolated in crystalline form, the hypothetical solanine group of glucosides is here referred to as "solanine."

To determine in what tissue the solanine is formed and stored, a number in the parenchyma and around the fibro-vascular bundles. By allowing the the most commonly employed procedures. Using such a method, ALBO reports (3: p. 199) that solanine is found relatively in abundance in active meristems of all parts of the plant, and that it disappears from inactive tissues. In the tuber he claims that it is

“particularly localized in the periderm (phellogen), although it is also found in the parenchyma and around the fibro-vascular bundles. By allowing the tubers to sprout, it is found very diffusive in the sprouts, and it is also observed in the pith and in the subepidermal cells of the roots.”

All such color tests involve the use of concentrated sulphuric acid (2), usually warmed. Under such conditions solanine is partially hydrolized to solanidine. Since solanidine also gives these color reactions, HANSEN concluded that such tests cannot be reliable. Color changes of anthocyanins when acid is added offer further complications.

To minimize these difficulties BREHMER (9) tried preliminary washing of cut sections with water, ether, and alcohol. Such a method should reduce the troubles from undesired colors, but it also allows leaching out of solanine. HANSEN tried to measure solanine by its haemolytic activity, but was not able to get quantitative results. FISCHER (18) showed that the rate of haemolysis varies greatly with the change in the acidity of the medium. Using buffered blood-gelatin strips a quasi-quantitative micromethod was developed by FISCHER and THIELE (19) which indicates solanine, as distinct from solanidine. They report no solanine in the periderm of the tuber, most of it being in the ten outer layers of cells of the storage tissue, but at any depth they find more near a sprout than farther away. In the sprouts they find both solanidine and solanine in the region outside the cambium, but only about  $\frac{1}{3}$  as much solanine and no solanidine in the central portion. They conclude that solanidine is first elaborated in an actively growing tissue and is later condensed with sugars to form solanine.

MORGENSTERN made a notable advance in gravimetric methods (34). His procedure involves extraction of a sample of ground tissue with water and warming the extract with acetic acid. After the filtrate has been evaporated to a syrup, hot alcohol is added. The filtrate is then evaporated to dryness, and is leached with hot acetic acid water. The filtered solution is heated with an excess of ammonium hydroxide. The precipitate is leached with hot alcohol, and the filtrate evaporated to dryness. The residue is dissolved in hot acetic acid water, and the solanine in the filtrate is precipitated with ammonium hydroxide and collected on weighed filter paper.

BÖMER and MATTIS have modified the procedure of MORGENSTERN somewhat. They recommend (8):

1. Extracting the ground sample four times with acetic acid water and combining the extracts.
2. Heating the extract with an excess of ammonium hydroxide and evaporating to dryness with diatomaceous earth.
3. Extracting the residue with three or four aliquots of boiling alcohol, alternated with repulverizing in a mortar.
4. Filtering the combined extract and evaporating to dryness.
5. Dissolving the residue in dilute acid and boiling the solution with an excess of ammonium hydroxide.
6. Filtering off and dissolving in hot alcohol.
7. Evaporating the filtrate to dryness and dissolving in dilute acid.
8. Reprecipitating with hot ammonium hydroxide and collecting on a weighed filter paper.

### Analytical considerations

Gravimetric methods are tedious and involve so many manipulations that loss of solanine is almost inevitable, in preparing a white precipitate. In a tissue bearing both glucoside and free alkaloid, each will be extracted and the weight of the final precipitate will represent the sum of solanine plus solanidine.<sup>2</sup> Since considerable amounts of the free alkaloid may occur in potato tissue (JORRISEN and GROSJEAN report (26) up to 0.15 per cent. in fresh sprouts), it would seem that a method of analysis would be desirable which would distinguish between the two substances and which would yield more accurate results than the blood, gelatin method of FISCHER and THIELE.

In solanine analysis, either hot ethyl alcohol or, more commonly, a dilute acid is used for extraction. Precipitation is accomplished by evaporation, or by addition of alkali. In these manipulations solanidine and certain magnesium and calcium impurities behave similarly to solanine, making purification possible only at the expense of loss of the product desired for weighing. Weighing introduces further difficulties, for solanine dries to constant weight very slowly. (A 5.3544-gm. sample of Merck solanine spread in a weighing bottle less than one centimeter deep dried to constant weight in an 80° C. oven at one-half atmospheric pressure only after twelve weeks. Moisture content 10.0 per cent.) A large number of filtrations is objectionable for aqueous solutions which have been made alkaline, since excess of alkali, and other factors not completely known, tend to produce a colloidal form of solanine which passes through the filter paper, or at best to produce a gel which clogs the paper, making each process tedious, and often requiring several papers for one filtration.

<sup>2</sup> To verify this, solanidine was prepared from Merck solanine by hydrolysis with 2 per cent. hydrochloric acid and decomposition of the crystalline hydrochloride with alcoholic potassium hydroxide. The base, recrystallized from ether, was found to be well soluble in alcohol and in 0.2 per cent. acetic acid although slowly so at room temperatures.

Extractions can be much simplified by allowing acid rather thoroughly to leach the ground tissue and then pressing quite dry in a hydraulic press. For this purpose a cylinder from an automobile engine, containing two piston heads (the upper one bearing a connecting rod and the other cut off at the second ring and perforated with several holes on the top) makes a satisfactory press cylinder, the pressed material being held in a linen bag between the pistons. A portion of the cylinder head provides support for the lower piston and drainage for the juice through a sparkplug hole into a beaker beneath. By this means it is possible to get out in one leaching practically all of the solanine extractible by the four separate processes of the BÖMER and MATTIS method.

To simplify the procedure and to avoid estimating solanidine as solanine, an attempt was made to estimate the extracted solanine volumetrically, obviating the necessity for weighing, and, more important, the necessity for eliminating the contaminating compounds whose solubilities are so like that of solanine. This is possible where solanine is estimated from the amount of sugar which is split off by acid hydrolysis, for none of these contaminants affect alkaline cupric ion. Because of the small amounts of sugar available in any one analysis, it was found expedient to use a modification of the PAVY reagent (36) with methylene blue as an internal indicator (30) to sharpen the end point. The alkalinity was increased in order to maintain at least one-half normal sodium hydroxide after addition of all the sugar solution, as seems advisable from the work of QUISUMBING and THOMAS (38), to keep a high ratio of cuprous oxide to sugar. The new procedure is simple and expeditious, save for the first filtration, which may be somewhat slow.

In the separation of tuberin from solanine, it is necessary that no solanine should be hydrolized by heating in dilute acetic acid. In a test of this, samples of one-half gram were boiled for one-half hour in 25 cc. of 0.2 per cent. acetic acid. No appreciable reducing ability developed, a confirmation of the work of ZWENGER and KIND (51), and of BÖMER and MATTIS (8). Indeed, solanine is unique in the conditions requisite for significant hydrolysis. COLOMBANO (12) claimed no appreciable hydrolysis at room temperatures for this concentration of solanine in 2 per cent. hydrochloric acid, even after a month's standing. The writer finds that after standing five months only 23 mg. of "glucose" appears under these conditions from a 500-mg. sample. In contrast, heating with 2 per cent. hydrochloric acid gives a fairly complete and rapid hydrolysis. HEIDUSCHKA and SIEGER (24) find over three-fourths of the solanine hydrolized in one-half hour at the boiling point.

### **Analytical procedure**

A sample of not much more than one kilogram of fresh tissue is ground in a Russwin mill, and the juice and solid material just covered by 0.2 per

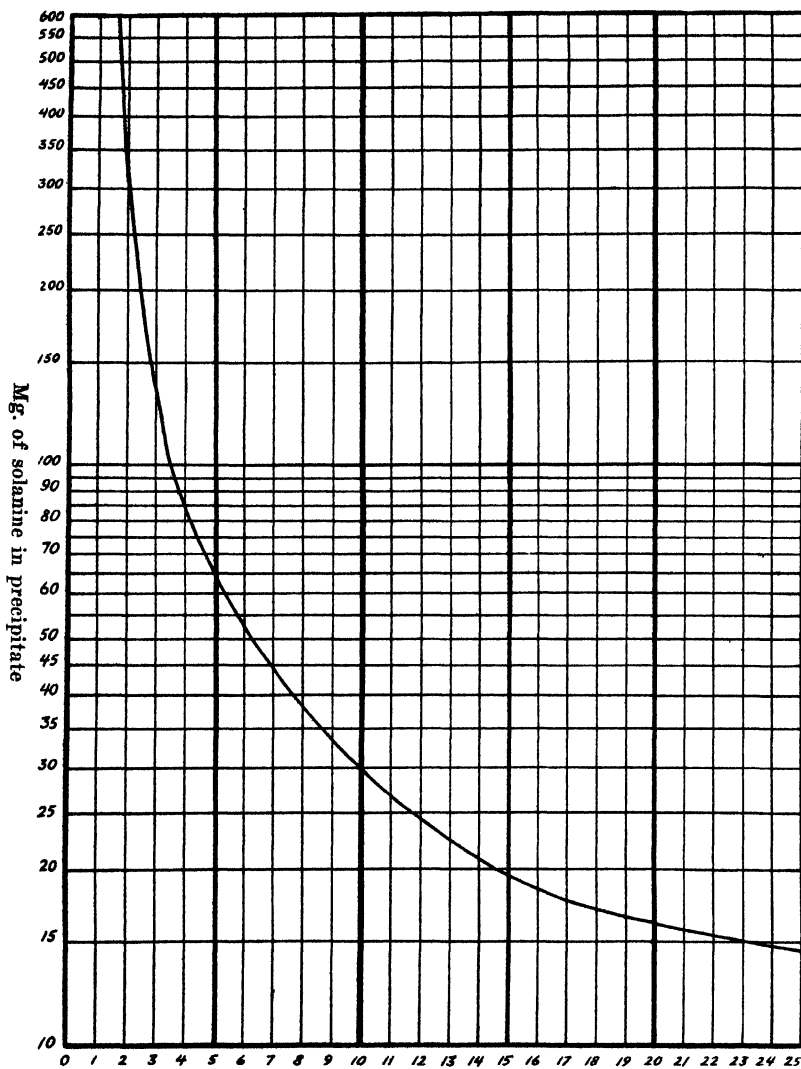
cent. acetic acid. After standing for about six hours, the liquid is strained through a linen bag, the residue being pressed out in a Carver hydraulic press, using a pressure of more than 35 kilograms per square centimeter. This expressed fluid is heated on a water bath to precipitate the tuberin, filtered through paper pulp, the pulp washed out with a few more cubic centimeters of acid, and the filtrate made alkaline by a few drops of ammonium hydroxide. This on further warming precipitates the solanine. It should be left in the evaporating dish overnight to allow large flocs to form. These are collected on a filter paper, in a Büchner funnel using suction, and washed with a few cubic centimeters of water containing a few drops of ammonium hydroxide. The volume of the filtrate is determined and the weight of solanine which it contains is computed, using the value 25 milligrams per liter (29).

For estimation, the precipitate is leached through the paper into a 50-ml. volumetric flask, by 25 ml. of 2 per cent. hydrochloric acid, and kept at a gentle boil on a hot plate for one hour. While hot, 10 ml. of 5 per cent. ammonia water is added, and after cooling, the whole is made up to 50 ml. with water. The reducing power of the filtered solution is determined by copper reduction.

The copper reagent consists of three grams of copper sulphate pentahydrate dissolved in 600 ml. of concentrated ammonium hydroxide, added to 1,000 ml. of Fehling's B, and the whole made up to 2,000 ml. with water. Such a solution shows no auto-reduction on standing. A 10-ml. aliquot is raised to boiling in a 300-ml. Erlenmeyer flask, with a drop of 1 per cent. aqueous solution of methylene blue. The flask is fitted with a two-holed rubber stopper, one hole for steam exit, and the other for the offset tip of a burette which is used to run in the sugar solution (the hydrolysate). Titration is complete on loss of the red color.<sup>3</sup> These titration values were standardized, using oven-dried samples of Merck solanine. Using the proportions indicated, at least 15 mg. of solanine should be present in the precipitate. Otherwise, the sugar solution formed is such a weak reducing agent that a large amount of solution is necessary to complete the titration. As it runs into the flask a significant amount of oxygen may also enter. Preferably a precipitate should contain less than one-half gram of solanine because of the danger that more concentrated hydrolysates may bump during the boiling. As shown by figure 1, intermediate values (20–100 milligrams) can be determined with greater accuracy.

Total solanine is obtained by adding the amount precipitated to the amount left in the alkaline filtrate, unprecipitated.

<sup>3</sup> The presence of ammonium ion in the sugar solution, added *during* the boiling, avoids one of the difficulties in the PAVY method, namely, precipitation of cuprous oxide if boiling is prolonged enough to drive off too much ammonia before the titration is complete.



Ml. of hydrolysate equivalent to 10 ml. of copper reagent  
 FIG. 1. Copper reduction values of solanine hydrolysates.

### Effect of light on tubers

Since solanine-rich tubers are frequently green, and since "sunburned" tubers are uniformly bitter and have an increased solanine content, the matter of tuber irradiation has received the attention of several investigators (7, 19, 21, 22, 34, 43). MÄRTIS found (7) that the metabolic activity was quite significant. Old tubers in storage showed very little response to

irradiation, while fresh tubers, when plowed out and exposed to direct sunlight, increased up to more than ten times their previous "solanin" content.

No investigation has been reported which has considered the relative significance of the various wave length bands of sunlight for solanine formation. Since the initial rise in solanine content commonly precedes visible chlorophyll synthesis (21, 22) it might be inferred that the former is not a consequence of the latter, but any convincing demonstration must involve the isolation of a selective radiation as a result of which no greening accompanies solanine elaboration. This paper describes an attempt:

(1) to increase the solanine content of tubers without chlorophyll elaboration; and

(2) to determine whether under equivalent intensities the wave lengths which chlorophyll ordinarily utilizes in glucose synthesis are the ones which are most effective in solanine synthesis.

#### EQUIPMENT

Seven bottomless wooden boxes were constructed,  $33 \times 33$  cm., 18 cm. high; the side walls were covered with white lead enamel, a good reflector for wave lengths from  $0.3 \mu$  to well beyond the visible (32: p. 88). The top of each box consisted of a set of four identical Corning glass filters,  $16.2 \times 16.2$  cm., whose margins were sealed with adhesive tape. An area of approximately 850 sq. cm. remained exposed to the radiation of the tungsten lamp overhead. These seven filters showed transmission peaks in various parts of the spectrum. The transmissions of these glasses were determined over the entire wave length range up to  $4 \mu$ , using a quartz spectrograph with sector attachment for the ultra-violet, a spectrophotometer for the visible, and a spectrometer with rock salt prism and thermopile for the infra-red.

Three additional boxes were prepared of sheet iron,  $38 \times 48$  cm., 30 cm. high, with tops which were tight-fitting removable trays 8 cm. deep. The bottom of each tray had a rectangular cutout  $32 \times 42$  cm. A sheet of ultra-violet transmitting glass was fastened into each tray bottom with pitch. A cooling coil of copper tubing, carrying a stream of tap water, was fitted just inside the tray wall. The coil and the metal portion of the tray were given two dippings in melted paraffin. Care was taken not to let paraffin overlap more than about 3 mm. onto the clear portion of the glass. Curved false bottoms were placed in each of the three boxes and two of the boxes were placed on platforms of unequal height, so that while the three box bottoms were horizontal, they were at sufficiently different heights to keep the three false bottoms as portions of one large cylindrical surface of 30-cm. radius, whose axis was the tube of an overhead Cooper-Hewitt mercury arc in Uviol glass, which had to be kept on a downslope for proper operation.



**TABLE I**  
**CHARACTERISTICS OF THE FILTERS EMPLOYED WITH MAZDA LAMPS**

FILTER			LAMP		RADIATION	
DESIG- NATION	MEAN THICK- NESS	APPROXI- MATE TRANS- MISSION LIMITS	DIS- TANCE	WATT- AGE	CHARACTER	DESIG- NATION
	<i>mm.</i>	$\mu$	<i>cm.</i>			
91B	7.62	0.365-4.2	53.6	100	Visible approximates arti- ficial lighting but weak in yellow band which distinguishes IV from V; infra-red intense; ultra-violet slight	I
			57.8	1000		VIII <sup>f</sup>
G90A	5.23	0.344-4.0	24.5	100	Visible like sunlight; infra-red considerable; ultra-violet slight	II
			51.8	1000		IX
G124JA	4.44	0.356-4.2	29.6	100	Visible largely green; infra-red slight; ultra- violet slight	III
			41.2	1000		X
G24	4.27	0.600-4.1	33.4	100	Visible only red and orange; (should be effi- cient for glucose syn- thesis); infra-red con- siderable; ultra-violet absent	IV
G34	3.96	0.520-3.6	44.1	100	Visible red, orange, and yellow (should be effi- cient for glucose syn- thesis); infra-red con- siderable; ultra-violet absent	V
G401CZ	4.85	0.460-0.620	34.0	1000	Visible green; infra-red intense; ultra-violet	VI
		and 1.1-3.6	23.8	1000	absent	XI
G53C	4.75	0.350-0.480 and 1.5-3.6	27.0	1000	Visible blue; infra-red slight; ultra-violet relatively intense	VII

Equal portions of the lamp were partitioned off for each filter by sheets of tin plate, as shown in figure 2.

Into one of these trays was placed a 5-cm. layer of an aqueous solution containing 290 gm. of  $\text{NiSO}_4 \cdot 6-7\text{H}_2\text{O}$ , and 83 gm.  $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$  per liter. The second tray contained a 5-cm. layer of an aqueous solution containing

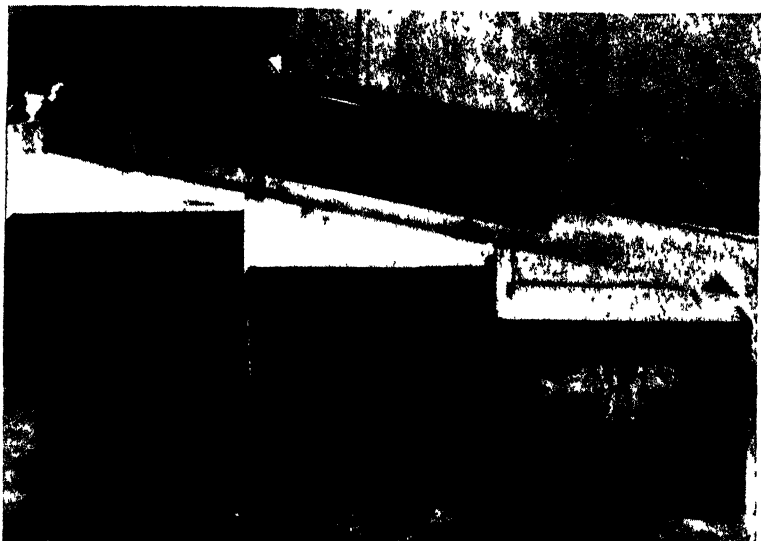


FIG. 2. Mercury arc lamp and metal boxes showing the three tray box tops with cooling coils and the four sheets of tinplate partitioning the lamp.

8.8 gm.  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  and 300 cc. of concentrated  $\text{NH}_4\text{OH}$  per liter. The third tray held a 5-cm. layer of an aqueous solution containing 200 gm. of  $\text{NaNO}_2$  per liter. Radiation entering these three boxes had to pass through the 5-cm. solution layer, the sheet of ultra-violet transmitting glass in the bottom of the tray, and in the case of the Cu filter an additional thickness of ultra-violet transmitting glass which was used as a cover. The transmissions of these filters was computed from measurements made on the solutions and on a sample of the glass, using a quartz spectroscope and a photo-electric cell. Figure 3 indicates the wide dissimilarity in opacity of these three filters. Although available emission data on the 450-watt Cooper-Hewitt arc<sup>4</sup> was limited to that furnished by BUTTOLPH (10) for a new lamp, it may be concluded that tubers under the  $-\text{NO}_2$  filter received almost no ultra-violet (radiation XIV), tubers under the Cu filter received long wave length ultra-violet and short wave length visible light (radiation XIII), and that the tubers under the Ni-Co filter received no visible radiation but did receive a much more intense exposure to wave lengths of about  $0.3 \mu$  than in the other two cases.

The tungsten lamps employed with the Corning glass filters were standard, internally frosted, 115-volt Mazda type C lamps operated at a potential of 113 volts (mean of a 100 hr. graphic record). When new, the energy distributions of their emissions were approximately as shown in

<sup>4</sup> Previously operated for about 1000 hours.

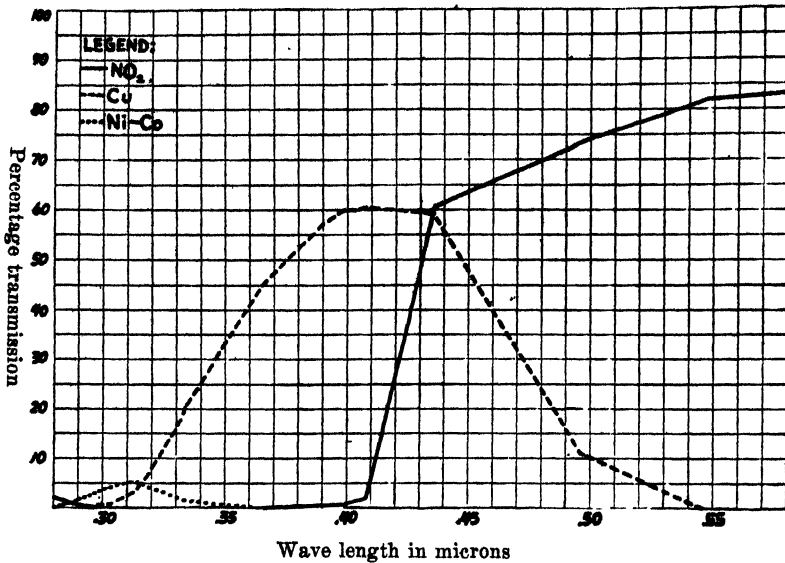


FIG. 3. Transmissions of the filters employed with the mercury arc.

figure 4. With use the emission decreases, but much more rapidly at the short wave length end of the spectrum. To minimize this error in the experiments reported, lamps were used for only 100 hours before replacing with new ones over filters which transmitted below  $0.45 \mu$ .

For each wave length the intensity of the lamp source was multiplied by the percentage transmission of the filter in question, and graphed, using a linear scale. Total energy of wave lengths shorter than  $0.69 \mu$  was read from this graph, using a planimeter. Knowing that the total radiation of a new 1000-watt lamp was 798 watts, and that of a 100-watt lamp 70.7 watts, and knowing that for the lamps used the intensity of the radiation was about

TABLE II

CHARACTERISTICS OF THE FILTERS EMPLOYED WITH MERCURY ARC LAMP

FILTER		RADIATION	
DESIGNATION	APPROXIMATE TRANSMISSION LIMITS	INTENSITY MAXIMUM	DESIGNATION
Ni-Co .....	$\mu$ 0.28 - 0.37	$\mu$ 0.30 and 0.31	XII
Cu .....	0.30 - 0.54	0.43	XIII
-NO <sub>2</sub> .....	0.40 - 1.0	0.55	XIV

21 per cent. greater than average, for the cone of light within  $48^\circ$  of the lamp axis (44: p. 22), it was possible to compute at what distance a lamp should be placed in order to have any desired intensity on the far side of an interposed glass filter. For computation, the distance from the filament to the center of one of the four glass plates was considered to be the average distance.

For radiations I, II, III, IV, V, VI, and VII these distances were made such that the energy of wave lengths less than  $0.69\ \mu$  getting through a glass filter would be 100 microwatts per sq. cm. for normal incidence. This

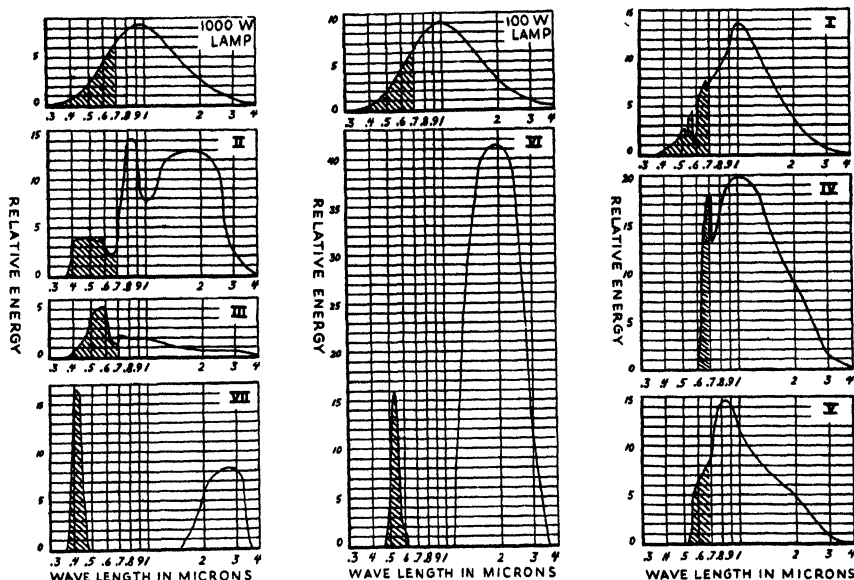


FIG. 4. Spectral energy distributions of radiations I, II, III, IV, V, VI, VII, and of the tungsten lamps employed. The equal energy areas are shaded. Wave lengths are shown on a logarithmic scale in order to make minor differences more apparent in the range which is significant for photosynthesis.

intensity value was chosen quite arbitrarily. The  $0.69\text{-}\mu$  limit was selected as approximately the upper limit of glucose and chlorophyll synthesis (13, 37, 39, 40). Tubers were placed in a single layer on the floor, covered with a filter box, and irradiated from an overhead lamp. Where 1000-watt lamps were employed, the reradiation from the glasses made a  $21^\circ \pm 2^\circ$  temperature control at the tuber level possible only after installing compressed air lines to ventilate the boxes.

For radiations VII, VIII, IX, X, and XI, the filter boxes were clustered on edge about a single 1000-watt lamp. In each box the tubers were held in a single layer between two pieces of  $\frac{1}{2}$ -in. wire mesh, and the box back

was covered with a light-tight pan. Two holes were cut through the bottom side and one in the top for the insertion of elbow pipe fittings so that ventilation could be accomplished through a suction manifold to a vacuum cleaner without entry of extraneous light.

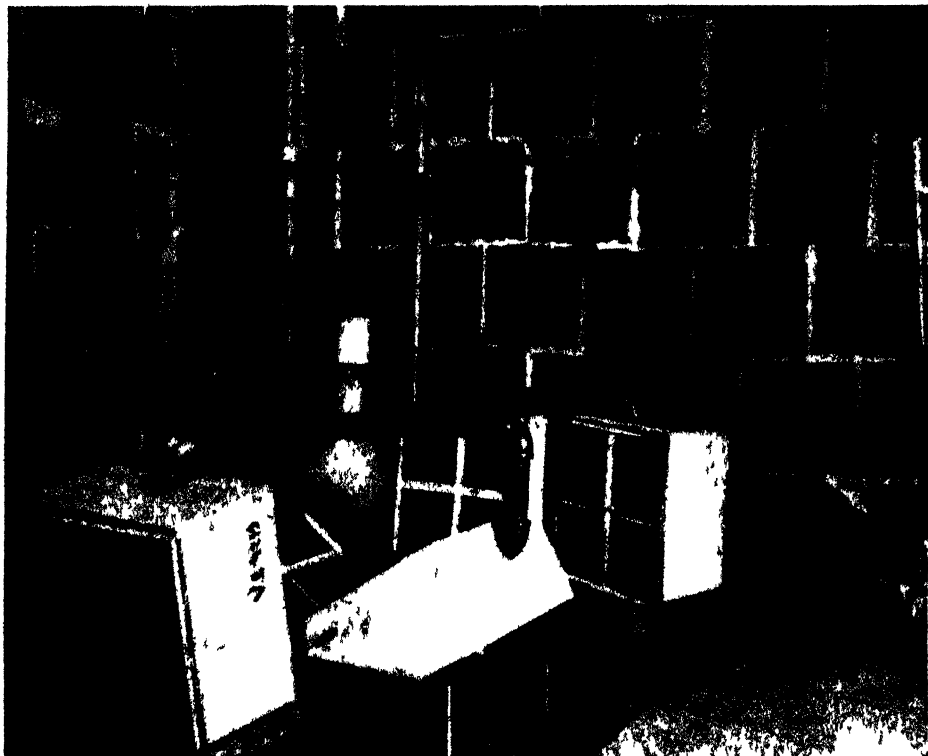


Fig. 5. Arrangement of Corning filters for radiations VII, VIII, IX, X, and XI.

From the results obtained with the previous irradiations, it seemed desirable to compare radiations which were of equal energy in the short wave transmitting band of filter G53C. At the 100-microwatt per sq. cm. intensity this was impractical for filter G401CZ since its transmission in this band ( $0.35-0.48 \mu$ ) was so low that it would have to be placed so close to the lamp that it would subtend too large an angle at the filament (with resultant gross uneven intensities getting through differing portions of the filters). Instead, the G401CZ filter was placed at a distance such that the lower half of its visible transmission band (below  $0.53 \mu$ ) let through the arbitrary intensity of 100 microwatts per sq. cm.

The angle of incidence of the light rays to the glasses varied from  $0^\circ$  to a maximum of about  $46^\circ$  for the closest placed filter. Within this range the reflection from glass surfaces is uniform (25: p. 1572) but the absorption

which occurs in the glass increases with the increasing angle of incidence. Since the transmission measurements were made only for normal incidence, the peaks in figures 4 and 6 should be somewhat more intense than shown.

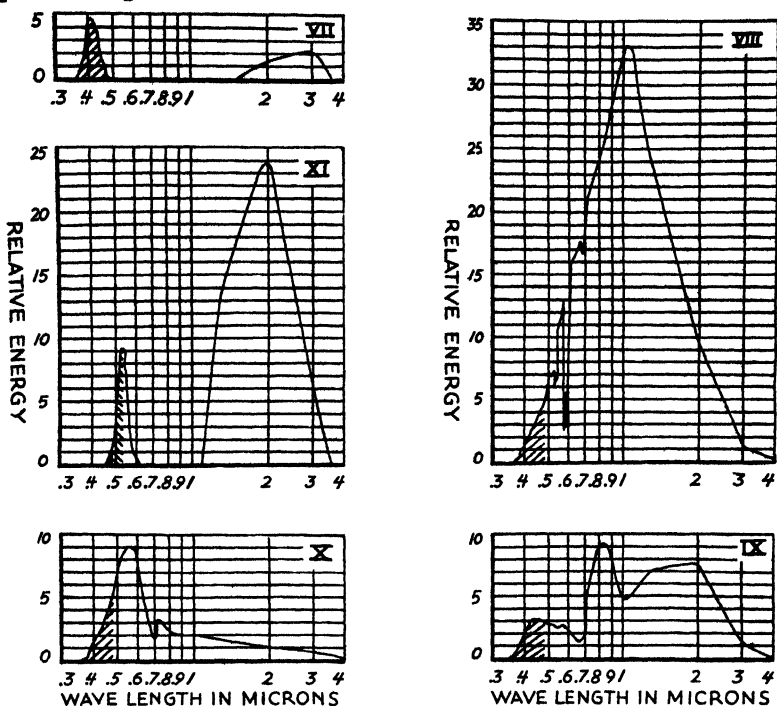


FIG. 6. Spectral energy distributions of radiations VII, VIII, IX, X, XI. The equal energy areas are shaded. Energy units are the equivalent of four of the energy units of figure 4.

Bliss Triumph potatoes were used. The "dormant" tubers were furnished by the State Agricultural Experiment Station at Spooner, Wisconsin. They were dug after sundown, in September, and kept continuously in darkness at room temperature for the two weeks prior to irradiation. This gave some opportunity for storage metabolism to be approximated. The "active" tubers, obtained in May from a dealer at New York Mills, Minnesota, were free from dormancy, having been dug by daylight the previous September and placed in a cool dark storage bin the same day. They were kept in the dark at room temperature for one month, the sprouts off, and the tubers irradiated.

## Experimentation

### SUNBURNED

"Sunburned" tubers (in which the washing away of soil has exposed them on one side to sunlight for an indefinite number of days) were har-

vested at maturity of the crop and immediately placed in a dark container. After four months' storage in the dark, the short (1-5-cm.) sprouts were removed and the tubers separated into two easily distinguishable portions, green and red, in every case cutting toward the center of the tuber from the boundary of the green zone. Both samples were intensely bitter.

Using the method of analysis described in this paper the solanine contents were found to be as shown in table III.

TABLE III  
SOLANINE CONTENT OF SUNBURNED TUBERS AFTER 4 MONTHS OF STORAGE

SAMPLE		SOLANINE			
DESIGNATION	WEIGHT	AMMONIA WATER SOLUBILITY	PRECIPITATE	TOTAL	PERCENTAGE CONCENTRATION
	<i>gm.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>%</i>
Red tuber	1001	11	20	31	0.0031
Green tuber	1015	9	24	33	0.0032
Sprouts	995	4	500	504	0.0507

#### DORMANT

Normal tubers were placed 30 cm. beneath the bare mercury arc lamp for a 20-day continuous exposure. Other tubers were exposed to radiations I, II, III, IV, V, VI, and VII, in each of which the total energy of wave lengths shorter than  $0.69 \mu$  was 100 microwatts per sq. cm.

A titration end point was not obtained for the hydrolisates from the precipitates of samples I, II, III, IV, V, VI, and check (indicating less than 10 mg. of solanine in any precipitate).<sup>5</sup> While the exact concentration in these samples remains uncertain, it is certain that the tubers receiving radiation VII contained at least 50 per cent. more solanine than the tubers under any of the other Corning filters. The analytical results are given in table IV.

#### ACTIVE

Normal tubers were continuously exposed for twenty days to radiations VII, VIII, IX, X, and XI, in each of which the total energy of wave lengths shorter than  $0.48 \mu$  was 100 microwatts per sq. cm. Other tubers were given twenty days of exposure to radiations XII, XIII, and XIV. Table V indicates the results obtained.

<sup>5</sup> However, there is no assurance that any precipitate contains solanine unless the titration is complete; the precipitate might be solanidine with calcium and magnesium phosphates only.

TABLE IV

SOLANINE CONTENT OF DORMANT TUBERS AFTER 20 DAYS OF CONTINUOUS RAYING

SAMPLE		SOLANINE			
DESIGNATION	WEIGHT	AMMONIA WATER SOLUBILITY	PRECIPITATE	TOTAL	PERCENT- AGE CONCENTRATION
	<i>gm.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>%</i>
Baro arc .....	437	25	22	47	0.0108
Dark (check) .....	276	5	<10	<15	<0.0026
I .....	601	7	<10	<17	<0.0028
II .....	543	4	<10	<14	<0.0026
III .....	625	3	<10	<13	<0.0021
IV .....	626	4	<10	<14	<0.0022
V .....	626	6	<10	<16	<0.0026
VI .....	799	3	<10	<13	<0.0016
VII .....	666	5	26	31	0.0047

For the tubers under the last three radiations, color distinctions were marked. Chlorophyll was absent in XII and XIII, and abundant in XIV. Anthocyanin was notable in XII, hardly noticeable in XIII, and not visible in XIV.

TABLE V

SOLANINE CONTENT OF ACTIVE TUBERS AFTER 20 DAYS OF CONTINUOUS RAYING

SAMPLE		SOLANINE				
DESIGNATION	WEIGHT	AMMONIA WATER SOLUBILITY	PRECIPI- TATE	TOTAL	PERCENTAGE CONCENTRATION	INCREASE OVER CONTROL
	<i>gm.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>%</i>	<i>%</i>
Beginning . . .	1725	14	30	44	0.0026	- 7
Dark .....	1197	6	28	34	0.0028	0
VII .....	1132	11	48	59	0.0052	+ 85
VIII .....	1096	10	93	103	0.0094	+ 236
IX .....	1128	7	96	103	0.0091	+ 225
X .....	1061	6	16	22	0.0021	- 25
XI .....	1158	5	25	30	0.0026	- 7
XII .....	1148	10	76	86	0.0076	+ 171
XIII .....	1135	13	34	47	0.0041	+ 46
XIV .....	1197	13	33	46	0.0038	+ 36

### Discussion

The concentrations reported here (by the volumetric method) are in general much lower than those obtained by gravimetric estimation of



"Solanin" (including solanidine) (7, 33, 34). BÖHME has reported that the normal content of potatoes (not sunburned) may be as much as 0.015 per cent., five times the amount found by the writer in very bitter, sunburned tubers, but BÖHME has reported other normal tubers with concentrations as low as 0.0020 per cent. GORTNER (20: p. 466) gives an average value for normal tubers of 0.0024 per cent., which agrees well with the < 0.0026 per cent., 0.0026 per cent., and 0.0028 per cent. values obtained by the volumetric method.

Perhaps more work has been done on anthocyanin formation than on any other type of glucoside synthesis which is induced by radiation. In a recent summary of the field ARTHUR (4: p. 1116) concludes:

"There is a general agreement among various investigators that the blue-violet and often the ultra-violet regions of sunlight are especially effective in anthocyanin production."

Since radiation XII was so much more effective than more intense radiations XIII and XIV, it may be concluded that the ultra-violet band is of more consequence than the blue-violet in the production of potato anthocyanin.

A comparison of qualitatively different light sources by equating total energies gives quite a misleading result where the significant radiations are only a small portion of the total energy, *e.g.*, the visible radiation of a Mazda lamp. This has been a serious error in the studies of KNIPE and MINDER (48) and of WURMSER (13). By equalizing energies below the  $0.69\mu$  limit, a better comparison was possible as to photosynthetic efficiencies. Greening, and glucose synthesis are most efficient in red-orange light (13, 47, 48). Since the most effective increase in solanine occurred upon irradiation by source VII it seems that a glucose synthesis cannot be a necessary intermediate. VII has no red, orange, or short infra-red, but neither has VI. VII lacks green, but so also does IV. The unique formation of relatively large amounts of solanine under radiation VII would not appear to be connected with the omission of any repressant rays, but rather with the presence of rays which induce the synthesis. Of the two possible bands, blue-violet—ultra-violet, and longer infra-red, the latter is ruled out since VII is intermediate in intensity between VI and III as regards these wave lengths.

To test whether the short wave band was responsible, the series of radiations with active tubers was tried. If short rays are effective, then increasing the intensity of I, II, and III to the same level as in this band (VIII, IX, and X) should also show increases in solanine content in tubers that are metabolically active. Whereas radiations I and II did not produce any notable effect on the solanine content of dormant tubers, the same sources were much more effective than VII on active tubers when the inten-

sity was sufficiently greater to give comparable amounts of the short end of the spectrum (VIII, IX). X did not show any increase. Whether green rays have a specific effect in the repression of solanine formation, as induced by short rays, has not been investigated, but the significance of short rays has been confirmed by the results obtained with the mercury arc. XIV contained almost no rays shorter than blue; it induced the least glucoside synthesis. XIII consisted of green, blue, and long ultra-violet; it gave intermediate effect. XII consisted of ultra-violet only, and was much stronger than XIII or XIV below  $0.32\ \mu$ ; it produced the greatest solanine increase. Incidentally it induced no chlorophyll formation.

This study has not been extended to the shorter ultra-violet or to the long infra-red beyond the emission limits of the Mazda lamp since such radiation probably cannot penetrate (11, 27, 45) to the tissues in which solanine is formed (19). It lumps as "solanine" all substances which precipitate out with the true solanine and which have—or which on acid hydrolysis split off—copper reducing groups. Solanine (17) and the glucoside of solanthrene (16) are included, if they occur, but so far as now known they are closely related to solanine in the metabolism of the tuber and they are present only in minor amounts.

### Summary

1. A new analytical method has been developed for the quantitative estimation of solanine in the presence of solanidine, based upon the amounts of sugars set free on acid hydrolysis.

2. Upon irradiation by a mercury arc in Uviol or by Mazda lamps, potato tubers increased in solanine. This was accompanied by the appearance of anthocyanin in the sprouts.

3. Wave lengths which are efficient for glucose synthesis did not induce a significant increase in solanine, but did result in an increase of chlorophyll.

4. Ultra-violet rays of about  $0.3\ \mu$  are effective for solanine formation but not for chlorophyll elaboration.

The writer wishes to express his gratitude to Dr. W. E. FORSYTHE for computing the emission of the Mazda lamps employed, to the Spooner Experiment Station for the tubers studied, to Dr. F. P. ZSCHEILE for measuring the aqueous filters, and to Professor C. A. SHULL for extending valuable suggestions and criticism.

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# METHODS OF RESEARCH ON THE PHYSICAL PROPERTIES OF PROTOPLASM<sup>1</sup>

WILLIAM SEIFRIZ

(WITH FIVE FIGURES)

Much excellent research has been done with a test tube and a Bunsen burner, but certain problems cannot be successfully attacked without the aid of intricate apparatus. It is the latter type of research, in so far as it applies to studies on the physical properties of protoplasm, with which this report deals.

Obviously, the first instrument with which the student of protoplasm must become familiar is the microscope. It had its beginning in the primitive use of lenses by the ancients. In the first book devoted exclusively to microscopical studies, "Micrographia," which was written by HOOKE in 1660, his compound microscope is illustrated (fig. 1).

It is not within the scope of this report to present a detailed description of the compound microscope. A knowledge of this instrument is extremely useful and it is suggested that the diagrams and descriptions of their optical systems appearing in the catalogues of leading microscope manufacturers be studied.

The microscope has a limit fixed, not by the magnifying power of lenses, but by the wave length of light and other factors which determine its *resolving power*. The highest powers of the microscope reveal objects which are about  $0.1\ \mu$  (0.0001 millimeter) in size.<sup>2</sup> Greater magnification could be obtained, but it would be of no use, because it would then be impossible to distinguish between two particles or lines which are closer together than the length of the wave of light used. It is not greater magnification, but

<sup>1</sup> One of the purposes of this committee is to make available those methods of physical measurements which have proven useful and have passed their preliminary experimental stages. However, all methods should be regarded as steps in the progress of research which undoubtedly will be modified and improved from time to time as new facts are discovered. Obviously these reports cannot be exhaustive treatments. They are intended to point out fundamental principles and include a bibliography which should serve as a starting point for those interested in a more comprehensive study. In the present report of the physical methods committee, organized for the committee by Dr. SEIFRIZ, the immediate objective has been to discuss briefly the approved methods and apparatus used in determining the physical properties of protoplasm and cell structure.—EARL S. JOHNSTON, Chairman.

- <sup>2</sup>  $\mu$  = micron = 0.001 millimeter  
 $m\mu$  = millimicron = 0.001 micron  
 $\mu\mu$  = micromicron = 0.001 millimicron  
 $\text{\AA}$  = Angstrom unit = 0.1 millimicron  
 $\mu\mu$  is often erroneously used for  $m\mu$ .

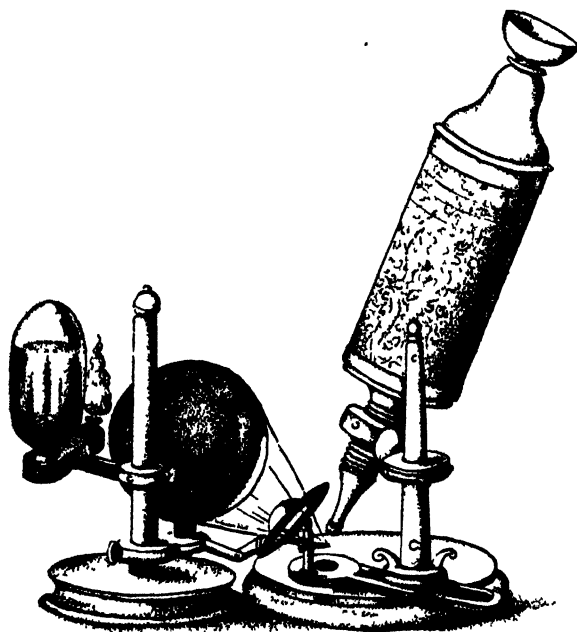


FIG. 1. The microscope of ROBERT HOOKE (1660). AJS

greater resolving power that is needed. Resolving power is the capacity of a lens to separate one line or point from another lying very close to it. Let us take a specific case: A diffraction grating, as used for the formation of spectra, may contain 1000 lines to a millimeter. Microscope lenses of the highest power easily distinguish these as individual lines clearly separated from each other. But if there are two lines where there was but one before, then each line will be separated from its neighbor by  $0.5 \mu$ . It would, in this case, be barely possible to distinguish individual lines if the microscope lenses and the eye of the observer were of the best. We can go no further with direct vision. As a result, efforts to increase the powers of the microscope have of late been directed toward methods of illumination.

### Illumination

Two lines of attack on the problem of microscopic illumination have been followed; either light of other wave lengths is used, or white light is applied in different ways. The light, other than composite white light, so far most successfully used is ultraviolet.

#### THE ULTRAVIOLET MICROSCOPE

The resolving power of a lens is proportional to its numerical aperture and inversely proportional to the wave length of the light used. Other

words, the greater the numerical aperture and the shorter the wave length of light, the smaller the structure which can be imaged by the lens. Written in the form of an equation:

$$\text{Smallest structure visible} = \frac{\text{wave length of light}}{\text{numerical aperture}}.$$

The average length of a wave of white light is  $0.55 \mu$  ( $5500 \text{ \AA}$ ); accordingly, a lens with a numerical aperture of 0.65 will reveal an object  $\frac{0.55}{0.65} \mu$  or  $0.85 \mu$  in size. This means that if the lens is used to examine parallel lines  $0.5 \mu$  apart, the image produced would not contain separate lines, the structure would not be resolved, and higher magnification would be of no avail.

The resolving power of a lens increases with the angle at which light enters it, and with the refractive index of the medium intervening between the lens and the object viewed. The increase due to the angle of the entering light is proportional to the sine of  $\frac{1}{2}$  of this angle. If the maximum angle at which light can enter is  $80^\circ$ , the sine of  $\frac{1}{2}$  of  $80^\circ$ , or  $40^\circ$ , is 0.64. If the medium between lens and object is water with a refractive index of 1.33, the resolving power will be 33 per cent. greater than if it is air, the refractive index of which is 1.00; and if the medium is cedar-wood oil with a refractive index of 1.51, the resolving power will be 51 per cent. greater than in the case of air. ABBE, German optician, introduced the term "numerical aperture" (abbreviated N.A.) to include both of these factors. The numerical aperture is the product of the sine of  $\frac{1}{2}$  the maximum angle at which light enters the objective, and the index of refraction of the medium between the front lens of the object and the cover glass. To increase the resolving power of a lens, we can (as the above formula shows) decrease the wave length of light, or increase the numerical aperture. The latter has been done by lens manufacturers as far as practicable, with 1.6 as the upper limit. As the numerical aperture is increased, chromatic and spherical corrections are more difficult, and the working distance of the lens becomes less. We then turn to the possibility of using light of shorter wave length. Blue light is shorter than the average (yellow) light of the sun. It should, therefore, increase the resolving power of lenses. Ultra-violet light, having shorter wave lengths than the blue, should serve the purpose still better. For this reason ultraviolet has been used, and the method has given rise to the *ultraviolet microscope*.

By using ultraviolet light with a wave length of  $275 \text{ m}\mu$ , as compared with  $550 \text{ m}\mu$ , the average wave length of white light, it is possible practically to double the resolving power of a lens. The theoretical limit of the resolving power of an objective of numerical aperture 1.40, is  $0.16 \mu$  for a wave length of  $450 \text{ m}\mu$ , and  $0.13 \mu$  for a wave length of  $365 \text{ m}\mu$ . This theo-



retical value of  $0.13 \mu$  is only slightly above the lower limit of microscopic visibility. An objective of numerical aperture 1.40, with the  $365 m\mu$  ultra-violet spectral line (of mercury) will give 19 per cent. more resolving power than the best microscopic system with white light. But there are difficulties. Glass is practically opaque to light waves of less than  $300 m\mu$ , i.e., to ultra-violet light; furthermore, such light is invisible to the human eye. The first difficulty is overcome by using quartz lenses, and the second by substituting the photographic plate for the human eye.

The ultraviolet microscope was presented to the biological world some thirty years ago by KÖHLER of the Zeiss scientific staff at Jena. The instrument has a potential resolving power twice that of the best optical systems using visible light, which means that it gives twice as much detail as do the best lenses with ordinary light. It also gives greater optical differentiation, i.e., greater contrast, for example, between the translucent, glass-like parts of a cell. It thus obviates the need of staining protoplasm, which is difficult of accomplishment in living matter. The method was originally intended for metallographic studies, but in spite of a promising future, little outstanding work was done with the ultraviolet microscope. LUCAS (14), of the Bell Telephone Laboratories in New York, has revived its use.

Ultraviolet light of a known wave length is obtained by passing light from a suitable source, such as a spark between cadmium or magnesium electrodes, through a quartz prism which resolves or breaks it up into its component wave lengths. Ultraviolet rays of about  $275 m\mu$  wave lengths are selected. As these are invisible, the optical image cannot be seen in the ordinary way. It must either be photographed or visualized on a fluorescent screen. The fluorescent screen commonly used is made of uranium glass, and is mounted interchangeably with a camera just above the microscope. Magnifications of 5000 diameters result in sharp brilliant images with a degree of resolution surpassing by far that achieved with any other known optical system. With such an equipment, LUCAS has taken some excellent photographs of living cells.

Any wave length shorter than the average of white light will increase the resolving power of a lens. Consequently, light *just beyond* the visible limit of the violet end of the spectrum (the visible spectrum stops at about  $390 m\mu$ ) should be better than white light, and it retains the advantage of white light in passing through glass. Ultraviolet of  $365 m\mu$  is, therefore, used with glass lenses.

The use of ultraviolet light for photographing living tissues is open to criticism because of the harmful effects which it may have on the cells. Certain microorganisms are destroyed almost instantly by ultraviolet light; others are mildly excited, and still others remain normal.

## VERTICAL ILLUMINATION

Direct light, *i.e.*, rays coming from below the microscope stage, is the time-honored method of illuminating transparent material through the microscope. Obviously, opaque material cannot be so viewed, consequently it must be illuminated from above. The types of apparatus designed for illumination from above are numerous; the following are representative. The illuminator of W. A. Beck (fig. 2) is a simple and inexpensive optical

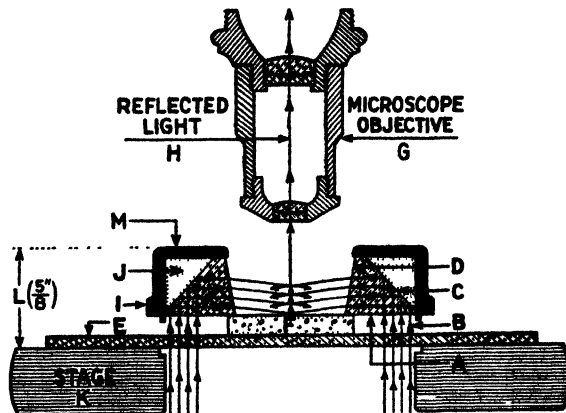


FIG. 2. The Beck illuminator. Light enters from below, is totally reflected by the prism C, and directed down upon the opaque material F.

system in which light comes from below, enters a circular glass prism from the upper silvered surface of which it is totally reflected to the opaque material lying in the center on the microscope stage. Similar, and as simple, is the "epi-mirror" of Carl Zeiss; it is part of the "epi-" equipment of Zeiss for dark-field illumination by incident light. One of the earliest of vertical illuminators is that invented by Silverman (manufactured by Spencer Lens Co.); instead of a reflector or prism, the light source is directly above the material surrounding the objective. Of a like design is the surface illuminator of Bausch and Lomb. It consists of a ring of electric lights surrounding the objective. Other more expensive devices for vertical illumination are the "Ultropak" of Leitz, and the "epi-condenser" of Zeiss. These latter instruments use light from a small and concentrated electric source placed close to the objective and entering it from above.

The vertical illuminator is a development of a metallurgical microscope which is used to advantage mostly in connection with the observation of highly-reflecting surfaces, such as those of polished and etched metals. Introduced by the metallurgist, the method has been adopted by the biologist (32) who finds it of advantage in studying not only opaque but also transparent material by viewing it in another light.

## DARK-FIELD ILLUMINATION

When FARADAY showed the Royal Society a suspension of colloidal gold, he told them that the solid particles of gold in suspension scattered light rays which strike them, so that a brilliant cone of light is produced. *Dark-field illumination* and the *ultra-microscope* are based upon this phenomenon. Colloidal matter is illuminated laterally against a dark background. Microscopically invisible particles thus become "visible" because of the light which they scatter. The direct beam of light from the source of illumination does not enter the eye of the observer; only the scattered rays from the particles are seen. The colloidal particles thus illuminated appear as brilliant spots against a black background. Two main types of lens systems are used to give lateral illumination—the cumbersome equipment known as the *ultra-microscope*, and the simple *cardioid condenser* or *dark-field illuminator*.

The development of the ultra-microscope is due to the Germans, SIEDENTOPF and ZSIGMONDY. The instrument designed by them is a large and expensive apparatus which others have attempted to simplify. This type of ultra-microscope is sometimes referred to as the slit microscope, the slit being a small aperture corresponding to the hole in the shutter of a darkened room into which a beam of light enters and illuminates the motes in the air. The ultra-microscope consists further of a series of lenses and apertures, apart from the microscope proper, which direct a light ray into the colloidal material and thus illuminate it laterally. The TYNDALL cone of a colloidal solution, as seen with the naked eye, is the total effect of the scattering of light by many particles, no individual particles being visible. In the ultra-microscope the individual particles are "seen" as centers of bursts of light. Thus viewed, a colloidal solution resembles the Milky Way at night, but with every "star" dancing about in active Brownian movement.

The simplest way to obtain a dark field with indirect illumination is shown in figure 3. Another simple method is the central stop diaphragm to be used with an Abbe condenser. It excludes all rays within the field of the objective's aperture. A dark field may also be produced quite easily by removing the top part of an Abbe condenser and substituting for it a dark-field element. The usual optical system for indirect illumination is the dark-field condenser. The same fundamental principles underlie this instrument as those of the slit type of ultra-microscope, namely, a black background, and indirect illumination. The light, however, enters the condenser from below as in an ordinary microscope.

There are several types and modifications, such as the *cardioid*, the *paraboloid*, and the *change-over* condenser. The first two names indicate the nature of the curve of the reflecting surface. The light rays strike and are

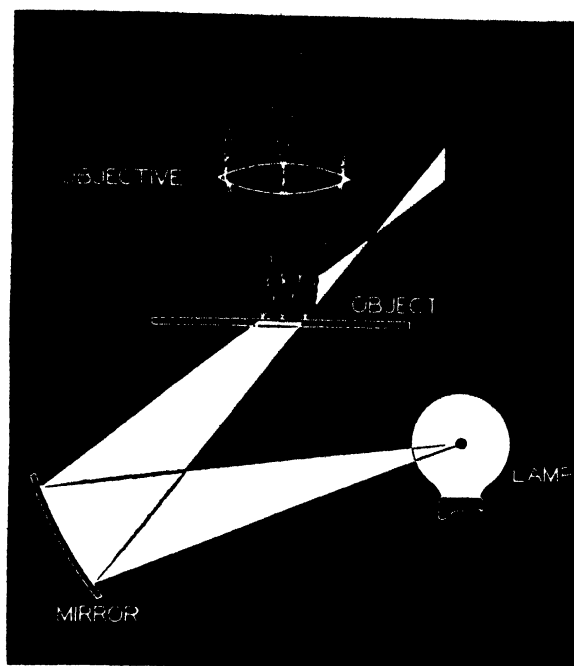


FIG. 3. A simple arrangement for dark-field illumination. (From Bausch and Lomb.)

reflected by two successive mirrored surfaces which direct and concentrate them at a point in the colloidal solution (fig. 4). Thus does the illuminating beam of light not enter the microscope, only the scattered rays from the colloidal particles being visible. The change-over condenser permits

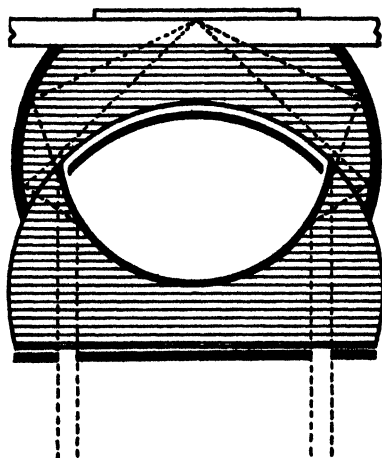


FIG. 4. The optical system of a cardioid dark-field condenser.

going from direct illumination, as in the ordinary microscope, to dark field without changing condensers.

A special dark-field condenser is manufactured by Bausch and Lomb which in itself takes the form of a moist chamber; it has been designed for work in microdissection.

#### THE SPIERER LENS

The SPIERER lens (26, 28), an ingenious development of dark-field illumination (o, fig. 5), is based on the principle that a colloidal particle scatters

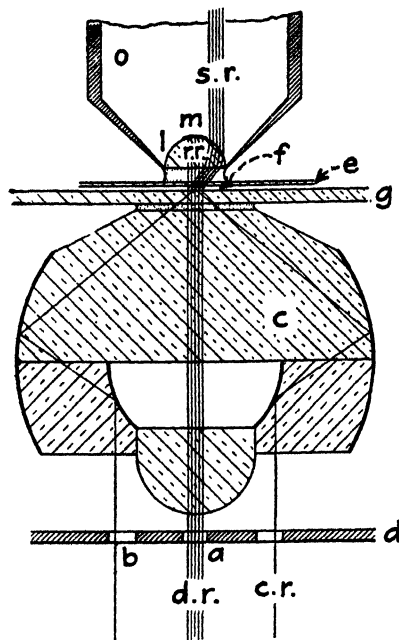


FIG. 5. The optical system of a SPIERER lens:

- o = objective
- m = Spierer mirror
- l = lens
- r.r. = reflected rays
- s.r. = scattered rays
- e = cover slip
- f = material
- g = slide
- c = cardioid condenser
- d = diaphragm
- a = aperture for direct rays
- d.r. = direct rays for Spierer lens
- b = slot for cardioid condenser rays
- c.r. = cardioid condenser rays

light unevenly, *i.e.*, there are more rays given off in one direction than in any other, namely, from that side which is away from the source of illumi-

nation. Degree of visibility of submicroscopic particles thus depends upon the angle of the illuminating ray. If a colloidal particle is viewed *toward* the source of illumination instead of at right angles to it, as in the slit ultra-microscope, or at 45 degrees to it, as in the dark-field (cardioid) condenser, the observer will then see the maximum amount of scattered light. A brighter picture, smaller particles, and a finer structure will consequently be discernible. In order to accomplish this, SPIERER placed a tiny metal (silver, gold, platinum, or aluminum) mirror (m, fig. 5) within an oil-immersion objective. This metallic reflector covers but a small part of the lens surface. Light comes directly from below, and passes through the colloidal matter. It then enters the lens, where it strikes the mirror from which it is reflected downward, thus illuminating the particles a second time from above. The first illumination is, however, the important one for it directs the maximum brilliancy of the ellipse (or rather the three-dimensional ellipsoid) of scattered light into the lens. In the usual dark-field condenser a less bright portion of the ellipse enters the objective.

The presence of a mirror in the lens prevents direct light from entering but does not hinder the entrance of scattered light around it. In order that only so much direct light shall enter as can be reflected by the mirror, the aperture of the iris-diaphragm below the microscope stage must be as small as the mirror. The SPIERER lens (o, fig. 5) is a dark-field system in itself; it may, therefore, be used with an ordinary Abbe condenser if the diaphragm is closed to a pinhole. But it is more convenient to use a special cardioid condenser (c, fig. 5) which has a fixed aperture (a, fig. 5) of correct size (*i.e.*, as small as the mirror, m, fig. 5). The use of a cardioid dark-field condenser adds still another ellipse of scattered light.

All dark-field methods of illumination depend upon diffraction phenomena for their usefulness. This is both an advantage and a disadvantage. The diffraction of light by ultra-microscopic structure makes that structure evident but the picture seen may not be an exact duplicate of the real one. It may be merely a diffraction image. This is not necessarily, nor always, true. One must, therefore, be particularly cautious in interpreting dark-field pictures. One can, however, always be certain that a diffraction image at least indicates that structure is there and usually it suggests the type of structure, if it is not an exact counterpart. With the aid of dark-field illumination, structural features in protoplasm not visible with direct light are made evident. Innumerable tiny particles in active Brownian movement are to be seen in protoplasm, as well as in the vacuoles of cells. TAYLOR (30) has determined the sign of the electrical charge on the ultra-microscopic particles in the protoplasm of a slime-mould; the particles were made visible by a cardioid condenser.

Ordinary light-field illumination usually shows the protoplasmic substratum to be homogeneous. Dark-field (SPIERER) illumination shows this apparently optically empty matrix to be a very fine emulsion consisting of two phases which have been termed *cryptoplasm* (hidden), *i.e.*, the optically empty background or matrix, and *phaneroplasm* (visible), *i.e.*, the emulsion globules. When once shown to be present by dark field, structures such as the fine protoplasmic emulsion can often be found with light field. SCARTH (22) has photographed the fine protoplasmic emulsion.

### Tandem and inverted microscopes

Biologists often try new combinations of lenses, sometimes to the distraction of the physicist who fails to find any optical reason for the new combination. But the biologist finds it works better that way and so goes happily on; thus two microscopes may be used in tandem, the second picking up the image of the first. This permits the use of a low-power lens on the first microscope and therefore greater working distance between lens and object. Greater magnification is obtained through the second microscope, but the resolving power of the complete system is determined by the resolving power of the first objective.

E. LEITZ has designed, at the suggestion of CHAMBERS, an inverted microscope: the objective occupies a position under the cover glass, while the fluid drop lies upon it. This establishes conditions which greatly favor the technique of micromanipulation.

### Vision at high speed

HARVEY and LOOMIS (12) devised a set-up which permits viewing a living cell while it is being centrifuged at high speed. The microscope, of course, is stationary. As the material passes under it, a light flashes on just for the moment so that one obtains an apparently continuous picture made up of many rapid successive exposures precisely as in the cinematograph. The gradual precipitation of granules due to centrifugal force can thus be observed while it is going on.

### The polariscope

The physical (optical) properties of crystals differ in different directions. An object seen through certain crystals (*e.g.*, Iceland spar) appears to be double. This is known as *double refraction*, and is believed to be due to the *polarization* of light. Ordinary light vibrates in all planes extending out from the point of propagation, but the vibrations of polarized light are restricted to one plane. If a crystal polarizes light, it will allow light already polarized to pass through in one plane only, and two crystals will permit polarized light to pass through only if their planes of polarization

are parallel. If two such crystals are crossed, more or less of the polarized light will get through, depending on the angle of rotation. When the crossed crystals are at right angles to each other, no light can pass through. Such crossed crystals when built for the study of polarized light are known as *Nicol prisms*; mounted in an instrument they constitute a *polariscope*. The presence of double refraction and the polarization of light by crystalline matter may be detected and studied with the polariscope. The two prisms, the *polarizer* and the *analyzer*, are inserted, the one below the microscope objective (usually in conjunction with the condenser) and the other above the ocular, the crystalline substance being placed between them on the microscope stage. Rotating one of the prisms, which is mounted on a scale, gives in degrees the angle of the plane of vibration. If the material is not crystalline there will be no decrease in light intensity as the Nicol prism is rotated.

By means of the polariscope, chlorophyll within a living cell has been found to be crystalline, and as it is fluid, it must be of the nature of a liquid crystal. FREY (10) has ascertained the crystalline structure of cellulose in plant tissues by means of the polariscope, and BAILEY and KERR (2) have added to our knowledge of the lamellar structure of cell walls, with a series of excellent photographs taken through Nicol prisms.

It is of fundamental importance to consider the possible crystalline character of protoplasm. The polariscope has not shown protoplasm as such to be anisotropic, but it has shown striped muscle and contractile tissue in general to be so (*i.e.*, not having the same properties in all directions, as is true of crystals).

### Microdissection

Microdissection and microinjection, with all forms of micromanipulation, are now included in the term *micrurgy*. The method, like all scientific discoveries, developed from primitive manipulative efforts on the part of pioneer workers. The first satisfactory instruments were constructed by BARBER (3) and by SCHOUTEN (23). BARBER built his pipette holder for the purpose of isolating single bacteria from cultures. The instrument is now replaced by several other types, each of which has its own peculiar advantages. The best known instruments which are commercially obtainable are the ZEISS-PÉTERFI (21) and the LEITZ-CHAMBERS (6) instruments. The TAYLOR (31) micromanipulator, privately manufactured, was an early design built for stability. A recent commercial model is that of FITZ (8), manufactured by Bausch and Lomb. A new but as yet little known model is that of DE FONBRUNE (37). It promises greatly to facilitate microdissection technique. It consists of a universal joint which controls three pistons operating against air. By means of three tubes, the pressure exerted by



the pistons is conveyed to a separate machine where three metal diaphragms, similar to those of aneroid barometers, are forced out or drawn in, and thus control, by means of levers, the rod to which the microneedle or pipette is clamped. The manipulator operates with remarkable precision, being wholly free from lost motion. The independence of operating and receiving mechanisms eliminates vibration. The hand of the operator and the needle-point move in the same direction and the operator's hand need never leave the one lever with which all movements are performed. DE FONBRUNE has also constructed an instrument for automatically drawing needles and pipettes under the microscope lens. In addition he has devised a simple method for protecting preparations of living material from dehydration without the use of a moist chamber. He simply places the material under an oil drop (nujol) either on the upper side of a slide or as a hanging drop. The oil is harmless, and lack of air has no ill effect for some time. Unfortunately the DE FONBRUNE micromanipulator is not yet commercially obtainable.

It is impossible to say which instrument is the best. The last mentioned gives promise of being so, but it has not yet been used by anyone except its inventor. Among the others, it is primarily a question of the instrument upon which one has learned. The LEITZ-CHAMBERS instrument is stable; the ZEISS-PÉTERFI has the greatest freedom of movement.

Accessories to micromanipulators are many. The fewer bought the better (certain ones sold are worthless). Exceptions are the needle-holders of Zeiss and the micropipette equipment of Leitz. Needles are best made by the individual investigator, because the type of needle should fit the work to be done, and the various types can be made only by hand. However, automatic devices such as drawing needles and pipettes are to be had (manufactured by E. Leitz). Microscalpel, microcauter, micromagnet, micro-electrode, and microthermocouple are some of the appurtenances to micromanipulation. The construction of each requires a special technique.

The problems of micrurgy are many, and some have been quite successfully attacked. Certain of them are of interest not only in themselves, but have important bearings on related problems, thus the elastic quality of protoplasm, ascertained by micromanipulative methods, is of value as an indicator (the best we have) of the structure and fundamental nature of protoplasm. Other problems which remain untouched, or as yet unsolved by this method, are the structure of the chloroplast and of other plastids (is the chloroplast a droplet or a sac?), the removal of a chloroplast from one cell and its injection into another cell, the reality and nature of spindle fibers, the physical properties of the huge, motile, male gametes of cycads, and the eternal problem of the outer protoplasmic membrane and the tonoplast which is not yet fully elucidated to the satisfaction of all.

### Electrical properties

The determination of electrical forces in organisms and cells is a fascinating study which is as yet in its infancy. These forces reach as high as a thousand volts, as, for example, in the ray-fish or electric eel, while that existing between the parts of plants, or between one cell and another, is of the order of 50 millivolts (0.050 volt). It is toward the measurement of these small electrical forces that our attention is here directed. This involves the use of microelectrodes (15) and a potentiometer circuit with amplifiers for greatest accuracy. Circuits for such measurements have been used and described by LUND (15) and his coworkers in the determination of potentials in trees.

Electrical investigations on cells and protoplasm require microelectrodes controlled by micromanipulative methods. Metal electrodes, even when of platinum, are likely to cause disturbances within the protoplasm; agar electrodes (agar with salt solution in a micropipette) are to be preferred. While in this review of methods we are concerned only with apparatus, it should be said that extensive work on other forms of potentials, namely, oxidation-reduction potentials, has been done with the aid of indicator dyes such as are used for pH determinations.

*Cataphoresis* studies have yielded some fundamental results on the electrical properties of living cells. The method involves subjecting suspensions of cells (or colloidal particles) to a difference in external potential; that is to say, the cells are placed in an electric field, and as all suspended particles, whether of metallic gold, oil, or living cells, possess an electric charge (with few exceptions), and, if free to move, they will migrate. The direction of migration is toward the pole of opposite sign to that of the charge on the particle. The rate is proportional to a number of factors including the potential of the external field and the potential (the interfacial potential) of the particles. These factors are all taken care of in the HELMHOLTZ formula for cataphoretic migration (37). With as yet no convincing evidence to the contrary, it can be definitely stated that all living cells are negatively charged when suspended in a solution similar (in regard to salt concentration and pH) to that in which the cells normally live. The interfacial potential of colloidal particles and cells in suspension is of the order of 30 to 50 millivolts; settling out (for example, of bacteria) occurs at about 11 millivolts.

The apparatus so far most extensively used in America for cataphoretic work is the NORTHROP-KUNITZ (20) model. It consists of a very shallow glass chamber open at the two ends to receive the electrolytic solution which connects the chamber with two poles of zinc immersed in zinc sulphate. A more recent model is that of ABRAMSON (1) which is less expensive and has the advantage of rigidity as it is constructed of one piece. The

NORTHROP-KUNITZ model is commercially obtainable. The ABRAMSON model is as yet only privately made.

To make simple and relatively crude cataphoretic observations is an easy task, but to make accurate measurements is quite another matter. The beginner is cautioned against the use of simple types of cataphoretic chambers which have been offered for sale. They are well-nigh useless and the results obtained with them are wholly unreliable. Practically all work purporting to show that cells are positively charged is due to the use of such, or home-made apparatus. Failure to measure pH is sometimes responsible for the trouble, for the sign of the charge on many suspended particles can be reversed by a change in the pH of the solution.

Simple comparative and reasonably accurate experiments in cataphoresis can be performed without calibrating the instrument, but for exact work this must be done. The best method for doing so is that of ABRAMSON (1).

Work in cataphoresis has yielded some of the most interesting and possibly far-reaching results of recent experimentation on the physical properties of cells. Only brief reference will be made to this work here, merely to call the attention of the student to articles wherein technique as well as results are described. Some of the earliest research on the cataphoretic migration of colloidal particles and bacteria was done by FREUNDLICH (9); then followed work by NORTHROP and KUNITZ (20) establishing the *critical* potential of bacteria. FALK (7) correlated the pathogenicity of bacteria with their potential. (This work may prove in part erroneous, but it was highly suggestive, pointed the way, and may yet be right to a degree.) More recent is the important work of MUDD (17) on sperms and bacteria, of ABRAMSON (1) on protein-covered quartz particles, and of MOYER (16) on latex. The last-mentioned investigation has made a fundamental contribution by showing that kinship or species relationship of Euphorbias is related to the shape of the mobility curves, and, primarily, to the iso-electric points of the latex particles.

### The centrifuge

The centrifuge has played a part in studies on protoplasm chiefly in regard to determinations of viscosity. The first work of this kind was done by NÉMEC (18). He found that changes in the viscosity of protoplasm occur during cell division. In the young dividing cells of the onion, bean, etc., resting nuclei are, by centrifuging, thrown against the apocentric cell wall, while spiremes and asters remain situated in the center of the cell. Metakinetic and telophasic figures are only slightly displaced. The most easily disturbed stages of cell division are those coincident with the formation of the cell wall. In other words, the protoplasm of the resting cell

preparatory to division is of low consistency; the protoplasm of the cell in mid-mitosis and immediately following is of high consistency, and the protoplasm of the cell in the last stages of division is of minimum consistency.

Recent developments have resulted in the construction of a small air-driven centrifuge which attains the highest speed yet known for centrifuges (5).

Centrifuging is but one of several ways of determining protoplasmic consistency. Other methods are microdissection (24), magnetic properties (37), the fall of statoliths (37), plasmolysis (34), and Brownian movement (4). Misunderstandings which have arisen, and a convincing clarification of them, have recently been set forth by FRY (11); he employed the centrifuge method.

All determinations of protoplasmic consistency by whatever method involve a consideration of the important question of the applicability of the single constant laws of Newton, Poiseuille, *et al.*, developed for true solutions and pure liquids, to systems such as protoplasm, the behavior of which is anomalous. The problem is an important one and involves recent rheological theories on non-Newtonian fluids (37). Investigations on the viscosity of protoplasm are many and the bibliography extensive; these have been reviewed by WEBER (33) and others (25).

### Photography

Photographs supply an authentic record of an experimental result. Photomicrography is now so highly developed a science and art that a discussion of it here would be wholly inadequate. A few remarks of interest and perhaps value may, however, be made. The small,  $4\frac{1}{2} \times 6$  or  $9 \times 12$  cm. camera which rests directly upon the microscope tube is very convenient, and excellent results can be obtained; but many workers still prefer to use the old-fashioned camera-box swung upon an upright support. The latter equipment is certainly the more flexible of the two, but it lacks one very convenient attachment of the former—the beam-splitter. It is a decided advantage to be able to focus through an ocular rather than upon a ground glass. Certain new but expensive photographic instruments combine the advantages of the old type camera-box and the new microscope camera with beam-splitter. The production of good photomicrographs is to a considerable degree dependent upon a correct choice of light filters. These can be had in considerable variety. The infrared filter is a recent addition and is to be used with special infrared sensitive plates. The photographer must try out the various combinations of lights, filters, and negatives suitable for his particular material.

The introduction of cinematography into microscopic work has yielded some fascinating results which are of real value to the research worker as

well as to the teacher or student. With the aid of moving pictures, details in mitosis heretofore unsuspected have been discovered. (A word of warning should be given in regard to the misleading impression created by speeding up films; pictures taken at the rate of one a second or one a minute then shown at the rate of 32 a second often give a highly entertaining but scientifically inexact impressions.) Motion pictures of tissue-cultures show the migration and division of fibroblasts and the movement of chromosomes in culture; others illustrate the development of the growing mammalian embryo from the one-celled stage to the sixteen-celled stage. Very striking are moving pictures taken with dark field.

For botanists, moving pictures have made their chief contribution in such studies as those on the mechanism of conjugation in *Spirogyra*, and on the operation of the animal-trap of *Utricularia*. These studies have been carried out by LLOYD (13). Through them is the method of movement of the male gamete in *Spirogyra* for the first time fully understood (motion is by contraction of vacuoles). A recent application of the cinematograph to protoplasmic studies has involved photographing protoplasmic streaming. Streaming protoplasm is always fascinating to watch, and becomes even more so when seen on the screen. But the method has a practical application; events which take place but once and too hurriedly to permit careful observation can be seen again and again if they have been photographed as moving pictures and are then shown at slow speed. The equipment necessary for taking moving pictures can be expensive but need not be. An essential feature is the beam-splitter so that material may be seen while it is being photographed. Other than this, any type of amateur camera for cinematography is all that is needed. The 8-mm. film is fairly satisfactory, but better results are to be had with the 16-mm. or professional 32-mm. film. The saving in cost of the 16-mm. over that of the 32-mm. film is to be considered in regard to the film, camera, and projector. Perhaps in no other way can an idea of the perpetual activity of protoplasm be so dramatically conveyed as through a moving picture of the streaming protoplasm of myxomycetes.

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# EFFECTS OF X-RAYS ON *ZEA MAYS*

MARY A. RUSSELL

(WITH EIGHT FIGURES)

## Introduction

Since the discovery of x-rays, their effects on both plants and animals have been studied as important biological problems. Many reports of early work are conflicting because quantitative results have been possible only in recent years, after methods of accurately measuring the dosage were perfected.

In the study of many general problems, plants are ideal material because of the large numbers which can be used and the ease with which environmental conditions can be modified or kept constant as occasion demands. Samples can be taken for cytological studies as the experiment progresses without detracting from the final results. Numerous reports on plant experiments have come from hospital laboratories where the work has been done with the conviction that many of the results will be applicable to problems of animal x-ray technique.

The work reported here was started for the purpose of finding out how *Zea mays* would react to x-ray treatment in various aspects of its growth and appearance, in order to use it eventually as a test material for studying effects of irradiation as they might be influenced by changing environmental conditions. *Zea mays* has been used occasionally for special radiation problems. STADLER (14) found it valuable for studies of mutations produced by x-rays because its genes had been more thoroughly mapped than those of many other plants. BERSA (2) reported *Zea mays* to be relatively more resistant to irradiation than many plants which he tested. He used it extensively for cytological examination of irradiated root tips.

## Materials and methods

The source of radiation was a standard air-cooled Coolidge tube. A constant potential of 180 kv. was used and the filament current was maintained at 3.7 ma. The distance from the center of the target to the material to be irradiated was 30 cm. The only filters were the heavy pyrex glass of the tube and the paper over the seedlings. The actual strength of the x-rays used was measured during radiation by the usual ionization-galvanometer arrangement, the ionization chamber having been calibrated previously with a standard chamber as described by TAYLOR and SINGER (17). The calibration of the apparatus gave a factor of 1.7 r units per centimeter of deflection of the galvanometer. Thus at a deflection of 15 cm. for 40 minutes, the dose



would be  $1.7 \times 15 \times 40$  or 1020 r. The ROENTGEN unit or "r" was defined at the Second International Congress of Radiology in 1928 as follows: "... the quantity of radiation which, when the secondary electrons are fully utilized and the wall effect of the chamber is avoided, produces in one cubic cm. of atmospheric air at 0° C. and 76 cm. mercury pressure such a degree of conductivity that one electrostatic unit of charge is measured at saturation current" (16).

Improved Golden Dent field corn was used in all experiments. The dry grains were planted between layers of moist paper toweling in glass chambers placed in a dark incubator at 24° C. After 72 hours most of the primary roots were slightly more than 1 cm. long. The grain was always placed in the germinating chamber in the evening at about the same hour in order to avoid differences in sensitivity due to possible natural mitotic rhythm. These precautions were also for the purpose of obtaining an equal degree of hydration in all experiments.

Seedlings were selected which had fairly straight roots 10 to 15 mm. long. A spot of India ink was placed exactly 10 mm. from the tip of each root and all future measurements were made from this point. In most experiments 20 seedlings were used in each group. The plants to be treated were individually placed on damp filter paper over the bottoms of glass dishes. The side of the grain where the shoot was about to emerge was always placed up in order that all of the embryos might be equally exposed to the x-rays. The dishes were closely covered with three layers of moist Scott Tissue toweling to prevent evaporation from the seedlings during radiation. The controls were kept under the same conditions outside of the x-ray apparatus.

Immediately after treatment the seedlings were placed either in pots of damp earth for observation in the greenhouse or in jars of loosely packed sphagnum to be put into the incubators for root study. The irradiated plants grown in soil had the normal greenhouse conditions of light and temperature. A large number of pots were prepared at one time with the same lot of soil to insure uniformity of nutrition.

## Results

### EFFECT OF X-RAYS ON AERIAL PARTS OF *ZEA MAYS*

The following observations were made on a typical series raised in the greenhouse. Groups of 12 seedlings each received doses varying from 100 to 5000 r. Up to the fifth day there was no difference in the heights of the shoots of the different groups, but on the fourth day close inspection showed that there was a delay in the bursting of the sheath or coleoptile in the heavily irradiated plants. The number in each group with the shoots visible at this time was as follows: controls, 11; 100 r, 7; 200 r, 8; 1200 r, 1; 1600 r and above, 0. On the sixth day all of the controls, 100, and 200 r groups had

burst the sheath as had also about 50 per cent. of the plants of the more heavily irradiated groups.

Measurements made 8 days after irradiation showed that all growth of the sheath had stopped both in the control and in all of the treated groups. The following figures indicate that the net growth of the sheath was not retarded by irradiation: average height of control, 22 mm.; irradiated with 800 r, 25 mm.; and 2000 r, 23 mm.

From the sixth day, the growth in height of the plants receiving doses of 1200 r and higher fell behind that of the controls. The upper limit of the amount of radiation which allows the majority of seedlings to unfold the first leaf was about 1200 r. When a dose of 800 r was applied, the growth rate during the first month was equal to that of the controls when only the height of the shoots was considered. When part of this 800 r group was removed from the soil and measurements made 12 days after irradiation, the roots had an average length of 207 mm. as compared with 238 mm. for the controls. After three months the tops of the remainder of the 800 r group were about two-thirds of the height of the controls. This may mean that in the case of the shoots there was a very long latent period before the effects of irradiation could be observed. On the other hand, it is possible that this later dwarfing was caused by lack of root development owing to x-ray injury, rather than a direct effect of irradiation on the tops themselves. After a dose of 1200 r, most of the seedlings died in two weeks, having grown little more than the group which received 2000 r, while the survivors, a quarter of the whole number, grew as well as the plants in the 800 r groups. At a dose of 1200 r the individual sensitivity seemed most apparent. The individuals in the groups receiving doses between 1400 and 5000 r showed little variation, either in length of roots, which averaged 54 mm. at death, or in height of shoots, which averaged 45 mm. at that time.

The leaves of plants which received a dose of 200 r and more showed many small areas of yellowish green between patches of normal color. Up to 400 r, the first three leaves were the only ones showing this chlorotic disturbance. With higher doses, which still allowed fairly normal growth of tops, a much longer time elapsed before normally colored leaves were produced. With very high doses the occasional plants which were able to produce one leaf, were decidedly yellow with very small areas of normal green color.

Leaves in the 800 r group, which were of normal length, showed a peculiar twisting and curling in some cases. The edges of the leaf were slightly inrolled and tended to move toward the under side. This condition persisted throughout the three months of the experiment.

#### SPECIAL ROOT STUDIES

The seedlings grown in sphagnum were used for observations of the behavior of roots after irradiation. Tests showed that the plants could be

carefully removed from the jars twice a day without affecting the rate of growth. It can be seen from table I that the final length attained by roots

TABLE I  
ROOT LENGTH ATTAINED IN SPHAGNUM AFTER KILLING DOSES OF X-RAYS

TEMPERATURE	DOSE IN R UNITS	HOURS AFTER TREATMENT	AV. LENGTH
°C.		hr.	mm.
22 .....	10,000	77	32
" .....	7,000	77	28
" .....	5,000	77	31
" .....	5,000	63	34
" .....	4,000	63	31
" .....	3,000	60	43
29 .....	3,000	50	41
24 .....	2,500	68	53
17-21 .....	2,000	134	51
22 .....	2,000	90	52
24 .....	2,000	70	50
24 .....	2,000	94	62
24 .....	2,000	85	51
29 .....	2,000	40	52
35 .....	2,000	45	44
36 .....	2,000	42	47
36 .....	2,000	43	41

after receiving doses of 4000 to 10,000 r shows great uniformity. The growth curves are also practically identical throughout this range. A dose of 4000 r would then appear to be near the limit beyond which further irradiation could be expected to have no observable effect, at least under these experimental conditions. As the dose is reduced below 4000 r there is a fairly regular increase in the rate of growth of the primary root after irradiation. Doses below 150 r produce no measurable differences between such treated seedlings and their controls when primary roots alone are tested.

A study was made to determine the smallest dose that would consistently produce a measurable difference between such treated seedlings and their controls. Preliminary observations showed that the rootlets growing laterally from the primary roots are the parts of the plant which first exhibit visible evidence of x-ray injury, although these rootlets cannot be seen at the time of irradiation. It has been found (table III) that, in all experiments at a given temperature, the time required for lateral roots to appear on normal seedling is remarkably constant. Table II shows that the time between irradiation and the first appearance of lateral roots steadily increases with the dose from 75 to 1500 r, which is near the upper limit of the amount

TABLE II

TIME OF APPEARANCE AND POSITION OF LATERAL ROOTS WHEN GROWN AT 24° C. AFTER X-RAY TREATMENT

DOSE IN R UNITS	APPEARANCE IN AT LEAST 33% OF CASES			AV. LENGTH OF PRIMARY ROOT	AV. DISTANCE OF FIRST ROOTLET FROM SEED
I	TREATED	CONTROLS	DIFFERENCE	V	VI
	II	III	IV		
	hr.	hr.		mm.	mm.
60	61	61	0	94	0
75	84	68	16	116	0
150	93	68	25	103	0
300	100	68	32	113	
300	87	63	24	122	6
600	94	67	27	98	
600	96	67	29	110	13
1,000	90	64	26	90	
1,000	114	63	51	65	25
1,000	160	68	92	98	20
1,500	160	67	93	65	31
1,500	160	68	92	73	20

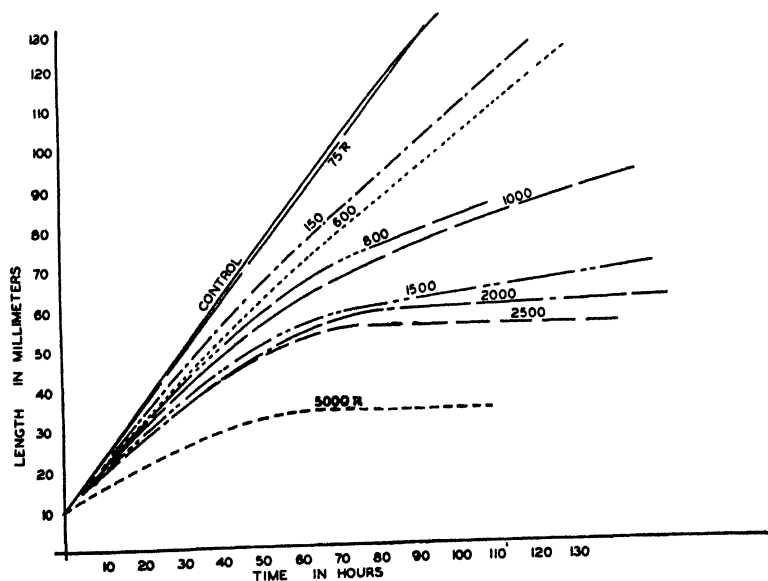


FIG. 1. Effect of various doses of x-ray on growth of primary roots of *Zea mays* in sphagnum at 24° C.

of irradiation that allows a few lateral roots to be formed on most of the seedlings.

Figure 1 shows that the growth rate of the primary roots treated with 75 r was identical with that of the controls, but the lateral roots in this irradiated group appeared 16 hours later than those of the controls. It has been stated above that 150 r was the smallest dose that could be depended upon to check the growth of the primary root in most of the corn seedlings.

Control seedlings grew at an increasing rate as the temperature was raised from 17° to 36° C. In these plants the lateral roots tended to emerge when the primary root was about 100 mm. long, irrespective of the time required to reach that length at various temperatures. Table III shows that

TABLE III  
TIME OF APPEARANCE OF ROOTLETS ON CONTROLS AT DIFFERENT TEMPERATURES .

TEMPERATURE °C.	TIME OF APPEARANCE OF ROOTLETS		AV. LENGTH OF MAIN ROOTS	
	hr.	hr. (mean)	mm.	mm. (mean)
37-39 .....	39	39	112	112
36 .....	38		106	
36 .....	36	37	82	94
35 .....	44		73	
35 .....	40		129	
35 .....	36	40	115	104
29 .....	40		95	
29 .....	36		91	
29 .....	43		104	
29 .....	39		108	
29 .....	47		104	
29 .....	45	42	96	98
24 .....	63		90	
24 .....	68		99	
24 .....	67	66	119	103
22-24 .....	96		122	
22 .....	97	96.5	122	122

with increasing temperatures the time required for the appearance of lateral roots decreases, while the length of the primary root at this time is very constant. For instance, in a group raised at 22° C., after the usual germination period at 24° C., the lateral roots appeared 97 hours after the first measurement. In another group raised at 35° C. the rootlets appeared after 48 hours, the average length of the primary roots being the same in each case. Figure 2 shows the very regular rate of decrease in time necessary for the appearance of rootlets as the temperature increases.

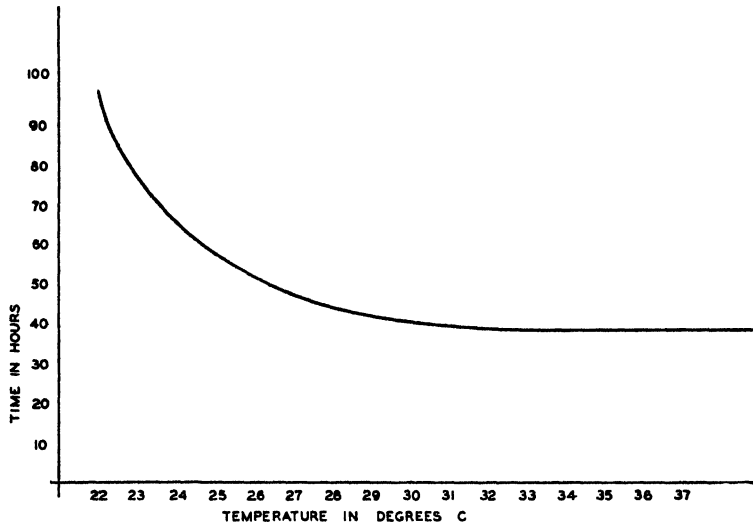


FIG. 2. Effect of increase in temperature on time required for appearance of secondary roots on seedlings of *Zea mays*.

Careful examination of the control seedlings showed that the rootlets first appeared on the primary root immediately below the grain in all cases, no matter how long their appearance was delayed by cold and the slow growth



FIG. 3. Section showing abnormal growth of secondary root after an x-ray dose of 1500 r.

accompanying it. It can be seen from column VI of table II that, when doses of 300 r and more were used, the average distance of the first lateral root from the grain increased from 6 mm. to 31 mm. after a dose of 1500 r. The roots grown in soil showed this same type of behavior after irradiation. With the higher doses the primary root had only a tassel of rootlets very close to its tip. Histological sections made from roots which had received a dose of 1500 r showed primordia of lateral roots in the region just above the level where rootlets were growing out in a normal manner. In some cases these primordial rootlets were subnormal in size and in a few examples they had turned either up or down, growing parallel to the longitudinal axis of the primary root, and they were entirely inclosed within its tissue. Figure 3 illustrates this condition.

#### EFFECT OF TEMPERATURE ON GROWTH AFTER X-RAY TREATMENT

Experiments performed to test the effect produced by keeping the corn seedlings at 6° C. during irradiation with doses of 200 to 1000 r and then allowing them to grow at 24° C. showed no differences between such treated seedlings and those similarly irradiated at room temperature. Following the failure to produce differences in sensitivity to x-radiation by means of low temperature during treatment, a number of tests were made to find out whether varying the temperature after x-ray treatment would affect the amount of injury due to irradiation. Since corn was found to thrive at temperatures of 17° to 36° C., it was possible to observe the behavior of roots whose growth rates varied widely according to the temperature selected. In the first experiment three groups of seedlings were irradiated at the same time with 2000 r, and then the separate lots were placed, with their controls, in three incubators at varying temperatures. Care was taken to measure the growth at very frequent intervals. Figure 4 shows the curves made by the

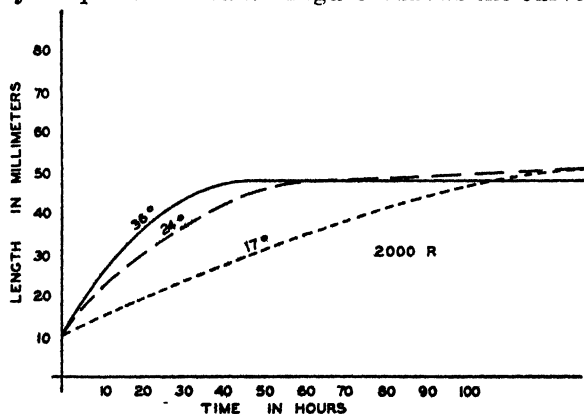


FIG. 4. Curves of growth at three temperatures after equal amounts of radiation. Length of life increases as temperature decreases.

growth of the primary roots of these seedlings exposed to 36°, 24°, and 17-21° C. after all were irradiated under exactly the same conditions. The shape of the curves is very significant, showing that the time of death was

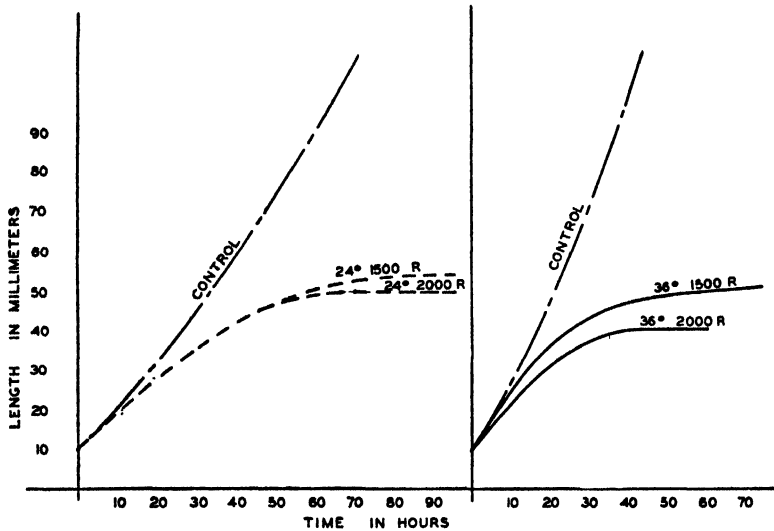


FIG. 5. Growth curves showing effects of different temperatures after doses of 1500 and 2000 r. Increasing doses decrease the latent period and the effect is exaggerated by increase in temperature.

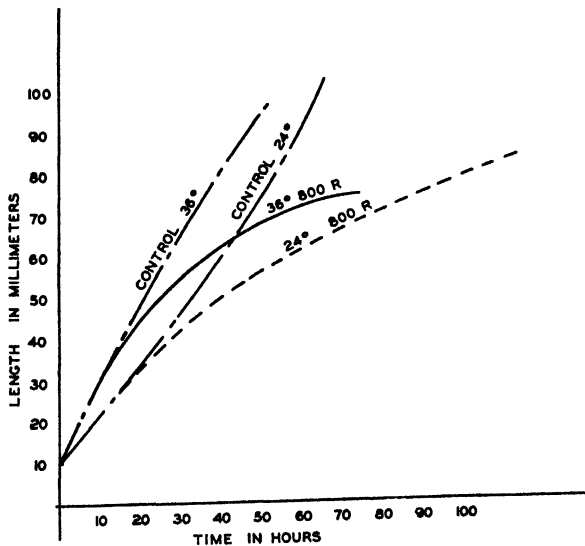


FIG. 6. Growth curves of primary roots of *Zea mays* showing effect of exposure to 24° and 36° C. after a dose of 800 r. At 20 hours the irradiated group at 24° C. shows no injury while at 36° C. roots are 4 mm. shorter than their controls.



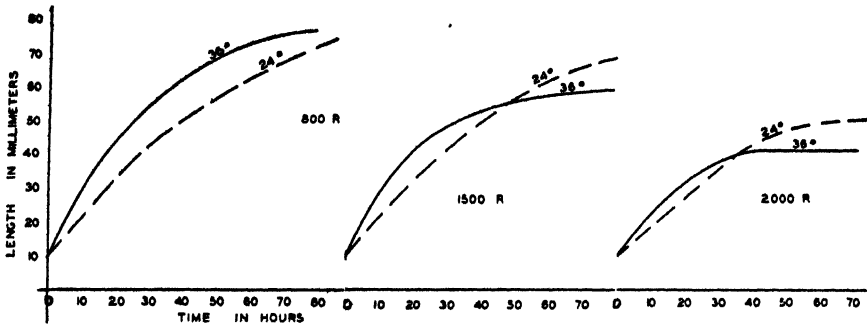


FIG. 7. Curves showing comparative effects of exposure to 24° and 36° C. after exposure to various doses of x-rays.

delayed as the temperature decreased. Similar experiments were performed by repeating the dose of 2000 r and also using 1500 and 800 r, in each case exposing part of the irradiated group to 24° and part to 36° C. with their respective controls at each temperature. Figure 5 shows that after both the 1500 and 2000 r treatments, the roots grown at 24° lived longer than those in the group at 36° C. Growth after 1500 r extends over a longer period of time at the lower temperature, and, also, the roots reach a considerably greater length before death when grown at 24° than at 36° C. Figure 6 shows that when the dose is lowered to 800 r, the irradiated groups grown at

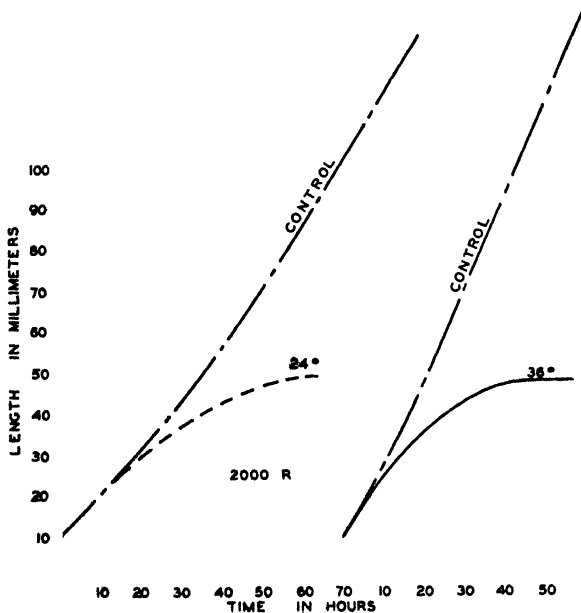


FIG. 8. Growth curves showing effect of temperature on length of latent period after an x-ray dose of 2000 r.

the two temperatures grow roughly parallel to each other, the 36° group growing the faster just as the 36° controls grow faster than the 24° controls. In figure 7 the curves of the growth following the three doses have been rearranged to show more graphically the effect of the two temperatures on each. The curves in figures 4, 5, 6, and 8 show that with all the doses used, the latent period was longer at the lower temperature. As the dose increases, the difference between the temperature effects is increased.

### Discussion

The coleoptile was the only part of the corn seedling which failed to show any effects of irradiation. CATTELL (3) reported it to be the least sensitive part of wheat, as after a dose of 1200 r the coleoptiles showed a reduction of 8 per cent. in height while the primary roots were reduced to 45 per cent. of the length of the controls. It would appear that the coleoptile of corn is less sensitive to irradiation than the coleoptile of wheat. The apparent lack of sensitivity to x-rays of this organ may be partly explained by the results of cytological studies of *Zea mays* by TETLEY and PRIESTLEY (18). They found that cell division stops in the coleoptile earlier than in any other part of the embryo. The fact that cell division has probably ceased long before exposure to x-rays may account for this organ showing no injury after doses which seriously interfere with the growth of parts of the seedling in which there has been rapid cell division at the time of irradiation.

SKOOG (13), using moving pictures to study the behavior of the coleoptile in *Avena*, found that its growth stopped when it was ruptured by the shoot pushing through it. If it is natural for the growth to continue only as long as the sheath is intact, and if the rupture is delayed when the shoot is stunted by irradiation, then this tendency towards excess growth in height may mask the effect of x-ray treatment on the corn coleoptile. The fact that the coleoptiles of treated corn seedlings were a trifle longer than those of the controls may indicate that the stunting effect of x-rays was a bit less influential than the opposite tendency towards increased growth.

The chlorotic disturbance noted in the corn leaves is typical of the results reported for many species. NOGUCHI (10) examined sections of leaves from irradiated seedlings of *Helianthus annuus* and found that the light color was due both to a reduction in the number of chloroplasts and a deficiency in their chlorophyll content.

The fact that irradiation in any amount between 4000 to 10,000 r failed to produce more stunting of the primary root than did 4000 r, shows that corn responds to x-rays in this respect in very much the same manner as does wheat. FRANCIS (5) reported that doses increasing from 3390 to 13,000 r fail to produce any increase in damage to the seedlings either in affecting the growth or in reducing the rate of respiration. Many workers have tried in vain to find a dose of x-rays strong enough to kill the plant immediately.

In an early experiment, where it was necessary to compare the effects of irradiation on two groups of seedlings which had been treated under different conditions, the time for comparison was chosen when both groups had lateral roots starting but those of one set were further developed than those of the other. Quantitative measurements were made at first by carefully cutting off the rootlets, obtaining the dry weights of those of the two groups, and then computing the average weight of the lateral roots of each group. This laborious method was discarded when further study showed that small differences in the amount of damage caused by irradiation could be measured both by noting the time at which lateral roots appeared and also by recording the distance below the grain at which the first rootlets appeared. The use of the time factor is the more exact of the two methods. It is necessary to decide on a given proportion, such as one-third, of the plants to be required to show rootlets before the time of appearance is recorded. This helps to prevent mistakes being made when the group contains one or more freak plants which, for some unknown reason, are unusually resistant. Such individuals have been omitted in computing averages when they were few in number and extremely different from most of the group.

When preliminary experiments have been carried out at the same temperature and near the dose to be used, the time interval during which very frequent observations are necessary to establish the time of appearance of rootlets can be fairly accurately predicted. In order to compare the sensitivity of two groups of corn seedlings, a dose of 300 r would have the advantage of yielding data both on the time of appearance and the spacing of lateral roots below the grain, and it would furnish this information in a shorter time than would be required for the same observations after higher doses. WIGODER and PATTEN (19) observed that lateral roots of *Vicia faba* were delayed in the time of their appearance and were formed more and more distally from the seed as the dose of x-rays was increased. It is therefore possible that this type of reaction could be expected in many kinds of plants and could be used as a general method for comparing sensitivity to irradiation between groups of the same species.

The fact that the appearance of the first lateral roots is delayed by irradiation might indicate that time is required for the repair of certain damage before these roots can be formed in a normal way. The few sections which were prepared from seedlings which had been irradiated with 1500 r showed no indications of lateral roots being formed in the region just below the seed, while in the region further down there were abortive attempts at lateral root formation followed by a region producing apparently normal rootlets. Since irradiation is generally considered to have an aging effect on tissues, the failure of the lateral roots to reach the exterior along the upper part of the root might be due to the same type of conditions which prevent secondary

root formation in old, tough roots. Further study may determine whether the rootlets in the x-rayed material changed the direction of their growth because of the mechanical difficulty of penetrating the outer layers of the primary root.

In normal seedlings there was a quite definite relation between the length of the primary roots and the time of appearance of lateral roots, but this relationship was upset in the irradiated groups. Here the rootlets tended to appear while the primary root was relatively shorter, but if the plant's main root never reached a length of 60–70 mm. it was unlikely that any rootlets would be formed. It may be possible that as the plant begins to suffer from the slow growth of the x-rayed primary root, the lateral roots tend to appear earlier in proportion to the length of the main root as an attempt at compensation for the lack of absorbing power of the main root. JOHNSON (7) reported that irradiated seedlings of *Helianthus annuus* lack ability to absorb as much water as their controls. This condition is all the more serious for the plant, because the aerial parts, not being stunted by irradiation to the same extent as the roots, are in need of a supply of water approaching that of a normal plant.

There are reports of several experiments on the effect of irradiating living material at low temperatures. CRABTREE and CRAMER (4) treated mouse tumor with radium at 0° C. and found that its sensitivity to irradiation was greater than when it was treated at room temperature. MOTTRAM (9) obtained similar results, using *Vicia faba* (a very small number of plants) treated with both radium and x-rays. On the other hand, HENSHAW and FRANCIS (6) failed to find any difference in the sensitivity of wheat seedlings when irradiated at different temperatures. The same type of experiment was tried in this laboratory with corn, with the idea that this plant, being more sensitive to cold than wheat, might give somewhat different results. The findings were negative.

It was found that growing corn seedlings at different temperatures following irradiation produced differences in the length of the latent period, the ones grown at the higher temperature showing the damaging effects sooner than those which were allowed to grow more slowly at the lower temperature. This held true for groups of roots which had received a dose of 800 r and grew in a manner very nearly like the normal. The effect on the latent period became more and more noticeable as the dose was increased to 2000 r. It would be of interest to test higher doses to determine whether a stage could be reached where the damage done by irradiation would be so great that a comparatively low temperature could have no effect towards lessening the amount of injury.

With all biological material the time required for the effects of irradiation to appear varies according to the dosage, the type of organism, and probably

many other factors. It is generally considered that biological changes are the result of chemical changes set in motion by ionization during the actual time of irradiation. Conditions which would speed up or modify the chemical reactions could be expected to shorten the latent period required for the results of the biological changes to appear. Heat increases the speed of most chemical reactions and does appear to shorten the latent period in the experiments reported here.

Although irradiated seedlings grown at 36° C. begin to show the effects of irradiation at an early age, they grow fairly rapidly until they suddenly die. When groups of irradiated plants grown at successively lower temperatures are compared, it appears that the slower the plants grow, the longer they are likely to live. However, the dose of 2000 r has set a limit, perhaps within a range of 6 mm., to the amount of growth possible following that amount of irradiation. The seedlings grown at each temperature group die when their primary roots have reached this limit no matter how long a time is required for each group to grow roots of that length. This limit may be determined by any one of several factors or a combination of them. A given amount of food material may be available for the building of new root tissue and irradiation may prevent the formation or transportation of more of that substance after the original supply is exhausted. If the root is growing rapidly, it will use up its supply and die sooner than a slow-growing root which uses up its food more slowly. This theory might help explain why the primary root can grow to the same length, when either 4000 or 10,000 r have been applied. If there is the same amount of food material available originally for both groups of roots, the 4000 r dose is just as effective as the 10,000 r dose in preventing any further growth after the original supply is exhausted. As the dose was reduced below 4000 r this food formation was apparently less and less disturbed. Since growth of the plant as a whole proceeds quite normally after a dose of 800 r, it may be assumed that there is early recovery if this amount of irradiation interferes with the food supply.

Another cause of death may be the accumulation of mildly toxic substances until they interfere with natural physiologic processes, or, as SCHWARTZ (12) suggests, the proteins may be giving rise to increasingly toxic products as their decomposition proceeds. Either of these conditions would be aggravated and cause earlier death with increase in temperature and the higher rate of metabolism accompanying it. The difference between the length of the latent period at different temperatures in the case of plants receiving 800 r, might be due to the difference in the rate at which toxic materials were formed. It is possible that some of these poisonous substances may diffuse out; this would help explain part of the recovery which takes place when the doses of radiation are not too heavy.

The comparative lack of irradiation injury shown in the early stages by plants grown at 24° C. and below might be due to the time element of delay

made possible by the use of lower temperatures. Several authors have tested the effect of allowing time to elapse between irradiation and the beginning of growth processes following it. STRANGWAYS and FELL (15) reported that when chick embryos were kept in the refrigerator for 24 hours after irradiation and then allowed to grow in incubators at their normal temperature, the injury due to irradiation was less than the injury suffered by embryos similarly irradiated but allowed to continue their normal growth rate immediately following exposure to x-rays. There may be a certain amount of recovery going on during the period before growth is resumed.

After living material has been irradiated there is a certain amount of residual effect which continues at a steadily decreasing rate. According to KINGERY (8), the rate of decrease of this effect with time follows a logarithmic curve. If growth processes are delayed for a time, the amount of residual effect will be growing less and less in the meantime. Therefore, when the rate of metabolism is allowed to return to normal there will be less damage done to the cells which are again beginning to divide than would occur if they continued dividing immediately after the irradiation period. In like manner, the long, slow growth made possible by low temperature could be assumed to reduce radiation injury by allowing more time for the reduction of the amount of residual radiation.

PACKARD (11) performed a set of experiments on *Drosophila* eggs which very nearly parallel the tests reported here on growing corn seedlings at different temperatures following irradiation. After the same dose of x-rays, groups of eggs which were incubated at 18° C. hatched in 80 per cent. of the cases, while in the group kept at 28° C. only 53 per cent. hatched. Low temperatures after irradiation also reduced the amount of injury shown by chick embryos in experiments reported by ANCEL and VINTEMBERGER (1).

The fact that corn seedlings, *Drosophila* eggs, and chick embryos all react to temperature effects after irradiation in the same general way would seem to indicate that temperature during this period controls one or more fundamental conditions involved in determining the amount of irradiation injury shown by the living organism.

### Summary

Seedlings of field corn were x-rayed with doses of 60 to 10,000 r and their subsequent growth observed under varying conditions. The results are as follows:

1. The height of the coleoptile was not affected by doses up to 5000 r which was the highest dose used in these particular experiments.
2. Shoots of plants which received over 800 r showed the effects of irradiation by delay in bursting through the coleoptile and by failure to reach the height attained by their controls during the time of the experiment.

3. All of the greenhouse plants irradiated with 200 r and more showed a chlorotic disturbance in the early leaves.

4. Leaves of all plants which received 800 r, and the small number of survivors from the 1200 r group, showed a strong tendency to curl their edges toward the under surface.

5. The rootlets growing laterally from the primary root were the most sensitive part of the seedlings. Doses of 75 r and higher delayed the appearance of these secondary roots increasingly as the dose was raised, while doses of 300 r and higher increased the distance between the grain and the first of these roots. Comparison of the time of appearance and spacing of these secondary roots is suggested as a method for comparing the degree of sensitivity to x-rays of various groups of seedlings.

6. Doses up to 10,000 r failed to prevent at least 20 mm. of root growth after radiation was applied.

7. Plants irradiated with doses between 800 and 2000 r showed a much shorter latent period before x-ray effects were measurable when grown at 36° C. after treatment than when grown at 24° C. and lower temperatures.

8. After a dose of 800 r corn seedlings showed the same amount of injury whether they were grown at 24° or 36° C. After 1500 r the 24° group grew faster than the 36° group after 50 hours, and the roots attained considerably greater length during their longer life. After doses of 2000 r the 24° groups surpassed the 36° groups after 35 hours and lived longer, but after this dose the final length was never more than 9 mm. greater than that of the 36° group. On the whole, lower temperatures after irradiation tended to reduce the amount of radiation injury.

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# LAG IN WATER ABSORPTION BY PLANTS IN WATER CULTURE WITH RESPECT TO CHANGES IN WIND<sup>1</sup>

J. DEAN WILSON AND BURTON E. LIVINGSTON

(WITH THREE FIGURES)

## Introduction

This paper presents a portion of the results of a series of wind-tunnel tests on water absorption, carried out in the laboratory of Plant Physiology of the Johns Hopkins University in 1924. We aimed to continue that study with more adequate control of air conditions and with other improvements in technique, but opportunity to realize that aim has not become available.

To make sure that the supply of water to the roots of our plants would always be unlimited, we employed solution cultures, which were usually supplied with SHIVE's well-known 3-salt solution of  $\text{KH}_2\text{PO}_4$ ,  $\text{Ca}(\text{NO}_3)_2$  and  $\text{MgSO}_4$ , in osmotic proportions of 5:2:3 and with a total osmotic value of 1.5 atm. Rates of water absorption and transpiration must consequently have been generally limited or controlled only by internal characteristics of the plants themselves (absorbing, conducting, and transpiring capacity) and by the current evaporating power of the aerial surroundings. Excepting in some special tests, these rates were never retarded by inadequacy of the external water supply at the absorbing surfaces nor by external suction, capillarity, or osmotic resistance to absorption by the root systems. Wilting of the leaves which could be induced by exposure to wind velocity of 12–15 mi/hr., with water-vapor-pressure deficit of about 15 mm. and air temperature of 28°–33° C., indicated simply that the absorbing and conducting capacities of the plant, taken together, were inadequate to deliver water to the foliage as rapidly as water was being lost through transpiration. Several species of plants rooted in well-watered soil also showed this type of wilting and LIVINGSTON has observed (5) plants of *Fagopyrum esculentum* L. in water culture to attain what must be regarded as "permanent wilting" in a sunny greenhouse, even without notable wind.

We were not now concerned with wilting, however, nor even with water absorption and transpiration under the influence of long-continued wind. In the experiments now considered the plant was subjected to artificial wind only long enough to attain a steady rate of absorption, after which the wind was discontinued and the absorption rate for calm air was soon regained.

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### Methods

Rooted cuttings were employed, mostly of *Salix purpurea* L. (basket willow). *Salix nigra* Marsh (black willow) and *Cephalanthus occidentalis* L. (button bush) were used in some tests. Basket willow is especially well suited to the preparation of a large number of closely similar rooted cuttings. Cuttings were made in late January, from shoots having a basal diameter of about 1 cm. They were usually 30–35 cm. long, but some were 120 cm. in length. The basal end of each cutting, protected by a suitable piece of tapered brass tubing, was thrust through the central hole of a large 3-hole rubber stopper, which seized the stem firmly in an air-tight joint. About 15 cm. projected on the lower side of the stopper. After removing the metal tubes, the cuttings were planted in moist sand in the greenhouse, to root and sprout. About 4 weeks later those which had developed satisfactorily were transferred to sheet-metal tanks (about 120 cm. long, 60 cm. wide, and 20 cm. deep) containing nutrient solution. The tanks, which stood in the greenhouse, were each provided with a sheet-metal lid with many perforations into which the rubber stoppers fitted, so as to keep the plants upright with their root systems in solution and their developing shoots and foliage in air. At the end of an additional 4-week period the plants were well developed and ready for use.

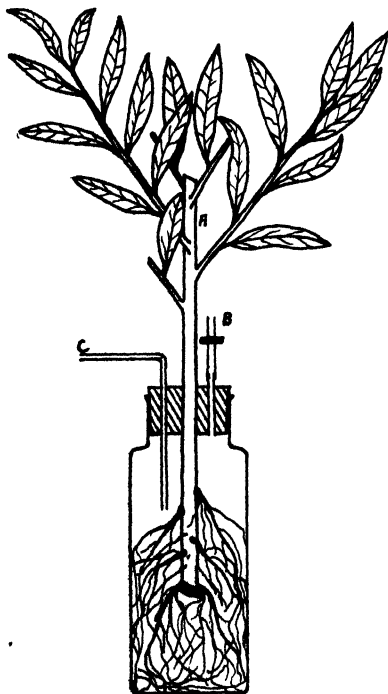


FIG. 1. Plant-culture bottle assembly.

When an experiment was to be started, a suitable plant was transferred from the tank to a large-mouthed bottle completely filled with about a liter of nutrient solution, a glass tube was tightly set into each of the unoccupied holes in the attached rubber stopper and the latter was firmly set into the bottle mouth, as shown diagrammatically in figure 1. Tube B could be closed at will by means of a bit of rubber tubing and a clamp of the Hoffman type. The assembly was then introduced into the wind tunnel and a potometer (shown diagrammatically in figure 2) was connected thereto by means of thick-walled rubber tubing, which joined tube C of figure 1 to one of the D tubes of figure 2. The culture bottles were kept closed for periods

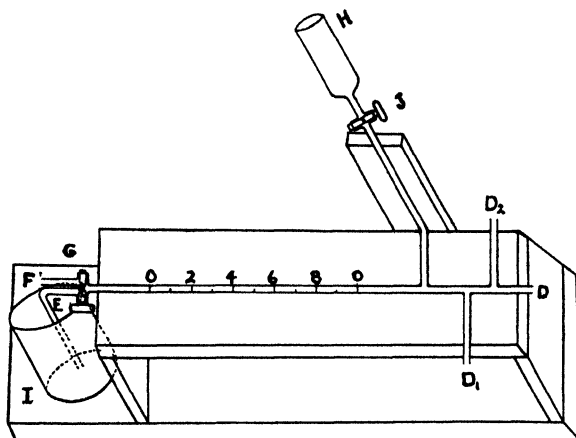


FIG. 2. Potometer.

no longer than were actually required for obtaining satisfactory potometer readings. Stoppers were lifted and allowed to rest on the bottle rims as much as possible and plants were regularly returned to the well-aerated stock tanks between experiments.

Standardized white spherical porous-porcelain atmometers of the LIVINGSTON form (7) were operated in comparison with the plants. The sphere was mounted on a vertical glass supply tube in the ordinary way, without a non-absorbing valve. The tube bore a 3-hole rubber stopper like those used on the cuttings, and the atmometer and its tube formed part of a bottle assembly similar to the one shown in figure 1. For atmometers, of course, distilled water replaced the nutrient solution. Each atmometer reading was reduced to the LIVINGSTON standard for this type of sphere by multiplying by the operating coefficient of the sphere used, which was 0.80 in every case.

Care was necessary to avoid the presence of undissolved gas anywhere in the whole bottle-potometer system, excepting the bubble index of the

potometer. Rates of removal of liquid from the culture bottle or atmometer bottle were measured, by 0.1-ml. or 0.50-ml. increments, by means of a stop watch. These rates were first recorded in terms of the number of seconds or minutes required for the removal of a single increment, but they were generally computed subsequently to terms of milliliters per hour (ml./hr.). Rates of atmometer absorption may be termed  $A$ , while those of plant absorption are termed  $P$ . Also,  $A_w$  and  $P_w$  may refer to rates secured with measured wind, and  $A_c$  and  $P_c$  to rates secured in the tunnel but without wind.

The potometer used (fig. 2) consists essentially of a horizontally placed piece of barometer tubing calibrated throughout a length of approximately 25 cm. and provided with a scale showing 1 ml. divided into twentieths. After the instrument had been connected to a culture or atmometer, distilled water which was placed in reservoirs I and H was allowed to enter through cock J so as to fill the entire system. With cock J closed, as well as any of the D tubes not in use, and with cock G open, application of a little suction at B (fig. 1) caused water to replace all undissolved air in the potometer-bottle assembly. During periods of experimentation, an atmometer was usually attached to one D tube, a culture bottle to another, and the third was closed with a piece of rubber tubing and a clamp. After the whole assembly had been filled, cock B was closed, and any water subsequently removed from the bottle by plant or atmometer was naturally replaced by movement from reservoir I. A small air bubble, to serve as index, was introduced through tube F. After each measurement, before the bubble reached the lateral tube leading to reservoir H, cock J was temporarily opened, to permit entrance of water from H, so that the bubble was driven back beyond the zero of the scale; the instrument was thus quickly made ready for the next measurement. The potometer tube was frequently cleaned with chromic acid cleaning solution, to avoid possible distortion of the air-bubble index.

It is generally impossible to expose in a room two or more stationary objects, such as plants or atmometers, so that they are subjected to really like sets of environmental conditions (4, 16). WILSON (17) observed great differences in evaporativity in different parts of the same greenhouse room; there is usually considerable air movement at different and fluctuating rates, and in various and changing directions, due to such causes as temperature differences or differences in air pressure between windward and leeward sides of the room. Furthermore, really calm air is practically impossible to obtain. GIDDINGS (3), and BLACKMAN and KNIGHT (1), who have discussed some of the problems involved in attempts to compare transpiration and evaporation in freely moving air, have described methods for avoiding some of the difficulties thus encountered. For such compari-

sons artificial wind from an electric fan cannot be employed without a suitable wind tunnel.

The tunnel used by us was similar in many respects to the one described by BLACKMAN and KNIGHT (1). It was horizontal and stood in a greenhouse room. It was mostly made of builders' wall-board ("celotex") supported on the outside by a light wooden frame. It consisted of a frontal portion about 75 cm. square in cross section and 2 meters long. Continuing this was a tapering portion 1.5 meters long and 45 cm. square at the smaller end, with a circular central opening just large enough to allow the propeller of a suitably supported 40-cm. electric fan to rotate in it, drawing air out of the tunnel. The top or ceiling of the frontal portion consisted largely of glass, to permit entrance of light. A suitable sliding panel in the floor of the frontal portion permitted the introduction of a plant or atmometer in the desired position and allowed the floor to be closed around the stem or supply tube. The bottle was supported beneath the tunnel and the potometer was conveniently placed outside, at a convenient level for reading. Air flow was regulated by means of a hand-operated rheostat which controlled the fan. The available range of air-flow rates was from about 1 km./hr. to about 26 km./hr. Rates of air flow were measured by means of a Mason anemometer placed in the center of the frontal section of the tunnel, and a thermometer suspended near by indicated air temperature. Wind velocities were expressed in terms of miles per hour (mi./hr.). The air of the tunnel around plant or atmometer was regarded as calm when the fan was not in operation, although there was surely always significant but unmeasured air movement under these conditions, especially upward and downward convection.

When a series of measurements was to be started, the rate of air flow was first adjusted; then the anemometer was removed from the tunnel, and an atmometer assembly was quickly introduced, the tunnel floor being closed around the atmometer tube. Several potometer measurements of atmometer absorption ( $A_w$ ) were then made as quickly as circumstances would permit, after which the atmometer assembly was replaced by a plant culture and a number of potometer readings of plant absorption ( $P_w$ ) were obtained. The fan was then stopped, and potometer readings with calm air were secured, first for plant ( $P_c$ ) and then for atmometer ( $A_c$ ). It required only a few minutes to replace the atmometer by a plant, and conversely.

### The potometer lag

It was found that the full effect of any pronounced alteration in air movement past plant or atmometer, which would measurably change the rate of transpiration or evaporation, was not immediately shown in terms of bubble movement; there was always some lag. After each change—

from calm to wind, from wind to calm, or from one measured wind velocity to another—it was necessary to secure several successive potometer readings throughout an adjustment period, until a steady rate of progress, corresponding to the new air conditions, had been attained. That period which is the subject of the present contribution, will be termed lag period. As might be expected, it was always shorter for the atmometer than for a plant under similar air conditions, and it varied in length according to time of day, weather, etc.

Since the liquid of the culture bottle or the atmometer sphere was continuous with the water column in the potometer tube, and since this liquid system was bound by rigid walls throughout, excepting at the menisci of the index bubble and at the open end of the tube, any movement of the bubble along the scale must have represented a corresponding change in hydrostatic pressure in the system; and that, in turn, must have represented removal of liquid from the bottle or from the cavity of the atmometer sphere, unless it were due to liquid contraction accompanying a lowering of temperature. The last clause refers to the familiar thermometer effect, which requires attention in all volumetric measurements of this sort, but temperature changes in the system could not introduce any considerable influence on bubble movement during the relatively short periods required for the timing of absorption increments of 0.1 ml. each. Volumetric rates of bubble advance along the graduated potometer tube are therefore taken as practically equivalent to concurrent rates of removal of liquid from culture bottle or atmometer sphere. These absorption rates were considered as controlled by current rates of transpiration or of evaporation, upon which air movement was effective, but the lag required attention.

For the sake of clearness it may be mentioned, without attempting a more thorough analysis here, that the rate of transpiration or evaporation at any moment is of course controlled partly by (a) internal characteristics (including temperature and exposure) of the transpiring surfaces or of the evaporating surface and partly by (b) the evaporating power of the surrounding air. Transpiration is of course subject to pronounced influences exerted by the absorbing and conducting capacity of roots and stems, as well as by structural characteristics of the leaves; but there is no internal resistance to evaporation from the atmometer sphere excepting what is introduced by the sphere wall—the atmometer tube offers no significant resistance to water movement (it has a bore of 6–7 mm.) and the instrument is without any resistance such as is due to the root system of a plant.

The water system within the pores of the atmometer sphere is apparently subject to slight readjustment whenever the rate of evaporation changes considerably, probably because of expansion or contraction of gas in small gas-filled spaces among the pores or because of slight outward or inward

movement or change in curvature of the submicroscopic menisci at the outer openings of the pores or because of both. Readjustments may also be due to volume changes in an air bubble in the sphere cavity, if one exists there. Aside from these suggested reasons for the apparent slight extensibility and elasticity of the atmometer water system, any change in evaporation rate should be accompanied by a corresponding change in the hydrostatic pressure within the sphere cavity, which should be transmitted almost instantaneously to the potometer tube, for we may be sure that no sensible change in the specific volume of water took place on account of such very slight pressure changes as might occur. The entire range of pressure fluctuation in our porous-porcelain spheres—mounted as they were without special precautions such as need to be introduced if one wishes to demonstrate negative pressures, as in the classic ASKENASY experiment—was less than one atmosphere; no truly negative pressure (*i.e.*, pressure with the *minus* sign, or traction pressure) was at any time developed, and the pressure differences here considered were consequently always within the limits of true suction, for which the liquid is at all times under positive pressure and is never “stretched.” In any event, the atmometer lag was generally short.

The plant differed fundamentally from the atmometer sphere and tube in that the liquid of the former was not held within rigid walls. With decreased hydrostatic pressure produced by acceleration of transpiration, for example, the volume of the liquid system of the plant might be considerably reduced without air bubbles forming in cells or in vessels free from undissolved gas, and of course gas bubbles might form or enlarge in some vessel segments. Thus, through bending of cell walls and corresponding minute but numerous pressure adjustments—in leaves, stem, and root—the plant might give off water temporarily at a rate more rapid than the concurrent rate of liquid absorption through the roots. If the transpiration rate is maintained after such an acceleration, these adjustments should soon terminate in a new set of pressure gradients throughout the plant, and then absorption and transpiration should again become equal and might continue so indefinitely. In a similar manner a reduction in transpiration rate (as when the air condition is changed from that of a considerable wind to that of calm) should be followed by a period of adjustment in the reverse direction, during which absorption should temporarily exceed transpiration. Such adjustment periods, with which students of plant transpiration and absorption have long been familiar, are probably the lag periods considered here. The liquid system of the plant thus acts, on the whole, as though it were extensible and elastic. In our tests, considerable increase or decrease in wind velocity apparently increased or decreased transpiration or evaporation almost instantaneously, but the lapse of the



lag period was required before the potometer readings came to represent the transpiration rate or the evaporation rate that corresponded to the new air condition.

The potometer lag cannot be related to any hydrostatic-pressure readjustments outside of the plant itself or outside of the atmometer sphere. As soon as a pressure change occurs in the culture bottle, or in the sphere cavity, it should be quantitatively transmitted to the potometer. Thus, although potometer readings taken within the lag period represent current rates of absorption by plant roots or by sphere, they do not represent precisely either current transpiration or current evaporation rates.

Plants of *Salix purpurea* and *Cephalanthus* were used in the representative experiments here described, the wind velocity employed being 4.4 mi./hr. unless otherwise stated. The length of the lag period for transpiration of both kinds of plant was found to be about the same, other conditions being alike. It was also about the same, other conditions being the same, whether the air change was in the direction from calm to wind or in the opposite direction, but in the latter case it sometimes appeared to be somewhat longer because the 0.1-ml. increments of absorption immediately following the stopping of the fan required much longer periods than were required when the fan was in operation. It was found that the length of the lag period was longer as the preceding steady rate of absorption for calm air had been slower, and conversely, thus showing a fairly clear diurnal march. In very early morning and late evening the period was longest, being shortest about midday, when the steady absorption rate for either calm or wind was at or near its maximum for the day. Furthermore, the amount of water absorbed during the lag period was greater as the period was shorter and as the absorption rate in calm air was more rapid, the average rate of water absorption during the lag period being most rapid about midday and least rapid in early morning and late evening. The length of the lag period was apparently not generally and consistently related to the accelerating effect of wind on transpiration.

For example, at about 6 A. M., 11 A. M., 4 P. M. and 9 P. M., on a clear day, the lengths of the mean lag periods for basket willow plants were respectively about 8, 3, 7 and 12 min., and the corresponding volumes removed from the potometer tube during the lag period were about 0.55, 0.75, 0.60 and 0.35 ml. Absorption rates in calm air were, respectively, 1.46, 6.0, 2.00 and 0.72 ml./hr., and accelerations induced by 4.4-mi. wind were 45, 30, 36 and 14 per cent.

For a second example, readings between 8 and 10 A. M. on three similar clear days, with air temperature about 20° and relative humidity about 66 per cent., gave the averages shown below.

PLANT USED	STEADY ABSORPTION RATE, CALM AIR	WIND ACCELE- RATION	CHANGE IN AIR MOVEMENT FROM	APPROX. LENGTH OF LAG PERIOD	APPROX. LOSS FROM POTOMETER DURING LAG PERIOD
	ml./hr.	%		min.	ml.
<i>Salix</i> .....	2.8	43	Calm to wind ... Wind to calm ... Average .....	7.3 6.8 7.1	0.35 0.45 0.40
<i>Cephalanthus</i> ...	2.4	88	Calm to wind ... Wind to calm ... Average .....	6.7 7.5 7.1	0.45 0.35 0.38
Atmometer ... ..	1.1	555	Calm to wind ... Wind to calm ... Average .....	1.3 5.6 3.4	0.15 0.1 0.1

As to the error that might be introduced if the potometer lag were neglected, a few numerical values may serve to show its order of magnitude; these are taken from the series of data on which was based the example just given. When a *Salix* plant was absorbing in calm air at a steady rate of about 2.8 ml./hr. the fan was started, giving a wind velocity of 4.4 mi./hr. Then the next three 0.1-ml. increments of absorption required, respectively, 1.84, 1.65 and 1.53 min.; thus the first reading after the fan was started showed a rate of 3.27 ml./hr., the second showed a rate of 3.64 ml./hr., and the rate shown by the third was 3.91 ml./hr. The steady rate for wind was about 4.00 ml./hr., and the first reading was therefore too low by about 18 per cent. For the reverse test, with the plant in wind and showing a steady absorption rate of 4.00 ml./hr., the fan was stopped, after which the next three potometer increments represented absorption rates of 3.6, 3.1, and 2.8 ml./hr., respectively, the latter being about the steady rate for calm air. The first reading after the fan had stopped was too high by about 30 per cent. These and other percentages of error that would be introduced by neglecting the lag following a change from calm to wind or from wind to calm are assembled below:

	CHANGE FROM CALM TO WIND	CHANGE FROM WIND TO CALM
<i>Salix</i> .....	18 per cent. too low	30 per cent. too high
<i>Cephalanthus</i> .....	25 per cent. too low	50 per cent. too high
Atmometer .....	Error too small to measure	13 per cent. too high

It is thus seen that the lag under discussion is apt to be of considerable importance, especially with regard to plant absorption. To avoid error due to it, the time periods required for the removal of successive 0.1-ml. incre-

ments from the potometer, after a change in air movement, were always recorded until a steady rate was attained, and that steady rate was then taken to represent the new air condition, as has been said. In this connection it should be noted that the hours of the day mentioned in this account represent the times when the respective tests were started. It usually required less than 20 min. to allow for the lag period in any test and to obtain the value of the steady rate; but when air humidity was high and rates of transpiration and evaporation were consequently slow, the time required for ascertaining all the several rates recorded for a given time was sometimes as long as 1 hr.

Data for an additional example of these lag relations are shown graphically in figure 3. Tests were made, using 0.05-ml. increments, at about 6 and 11 A. M. and at about 4 and 9 P. M., at which times the acceleration due

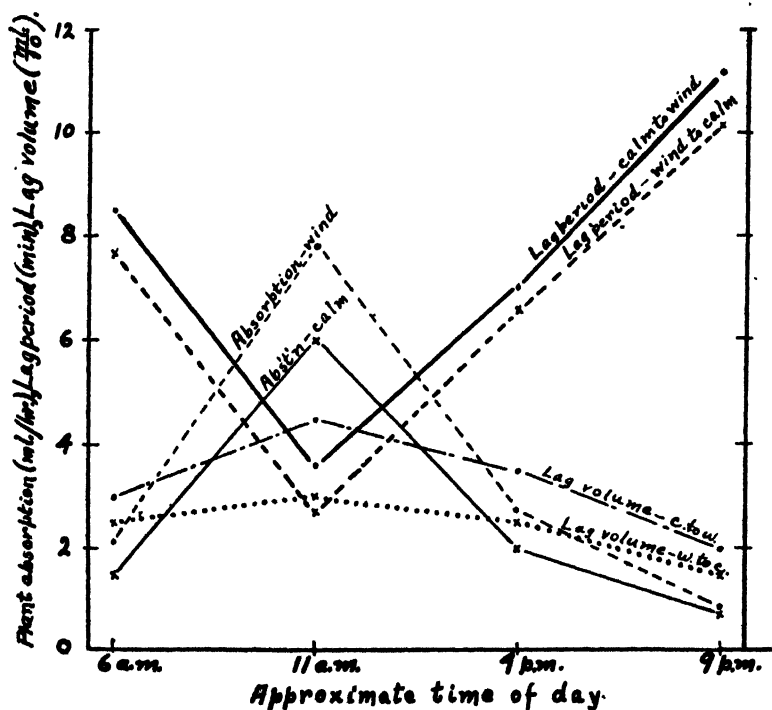


FIG. 3. Rate of absorption of culture solution by a willow plant in calm and wind at different times of day, the corresponding length of the lag period in a change from calm to wind and wind to calm, and the volume of solution absorbed during these periods.

to wind amounted to 45, 30, 36, and 14 per cent., respectively. The plant used was a basket willow and the wind velocity was 4.4 mi./hr. The steady absorption rates, for calm air and wind (the latter being of course the greater) are seen to have been maximal at about 11 A. M., which is also true

of the lag volume (amount of absorption occurring during lag period), but the length of the lag period is seen to have been minimal at that time. In this instance the lag period and the lag volume were a little greater for the change from calm air to wind than for the change from wind to calm air.

MARTIN and CLEMENTS (10) found that a pronounced and rapid initial acceleration of transpiration occurred when their weighed sunflower phytometers were subjected to artificial wind after a period of calm. Their first measurement of the rate of water loss was not made until 15 min. after the fan had been started, but they were convinced that the maximal transpiration rate had been attained and passed in that first period. It seems probable that their plants, with roots in moist soil, did not attain their maximal rate of water loss more quickly than did our plants, with roots in solution. The lag period for basket willow, with which most of our tests were made, was usually less than 10 minutes.

It seems clear that, as has been remarked, the potometer lag for our plants represents hydrostatic readjustments of some sort within the plant body and that the lag period represents the time required for the propagation of a hydrostatic-pressure change from transpiring membranes in the leaves to the external solution bathing the absorbing membranes at the root surfaces. These readjustments probably occurred mainly in cortical tissue, especially in the leaves, in the experiments thus far considered; they might, however, occur in cortical tissues elsewhere in the plant, as in stem and roots, and of course they might occur in the xylem vessels.

To gain some insight concerning stem-length influence on the potometer lag, a series of comparisons was made between long-stemmed and short-stemmed basket willow plants. The short-stemmed plants had stems about 30 cm. long, while the long-stemmed plants had stems about 120 cm. long. These tests on stem length were otherwise regular in every way and all plants were essentially alike with respect to foliage and root systems. A wind velocity of 6.6 mi./hr. was used in comparison with calm air. Some representative results follow:

	SHORT STEMS (30 cm.)	LONG STEMS (120 cm.)	
Steady absorption rate in calm air (ml./hr.) ..	4.9	4.7	
Acceleration due to wind (per cent.) .....	34	36	
Length of lag period (min.) {	Change from calm to wind .....	7.1	8.2
	Change from wind to calm .....	7.1	8.0

The lag period for the 120-cm. plants was thus about 14 per cent. longer than for the 30-cm. plants and it seems clear that this difference was due to difference of stem length. Supposing that stem resistance to propagation of a hydrostatic pressure change from leaves through roots was proportional to stem length, and supposing that resistance due to foliage, or to foliage and roots taken together, was alike in both cases, then the equivalent of 14 per cent. of the shorter lag period (or 0.98 min.) may be considered as having been due to 90 cm. of stem in the long-stemmed plants. Accordingly, the length of lag period due to leaves or to leaves and roots may be taken as 6.7 min. in both cases, to which is to be added, for stem influence, 0.33 min. (short stems) or 1.33 min. (long stems). This reasoning leads to the plausible supposition that only about 5 per cent. of the shorter period and only about 16 per cent. of the longer period is to be attributed to stem resistance. On this basis, the stems would apparently have had to be about 630 cm. long to make the stem lag as great as the leaf-root lag. Of course these tentative computations indicate only the general order of the magnitudes considered and the percentages shown would be expected to fluctuate with the transpiring capacity of the plant considered, which was apparently about the same for both kinds of plants in these tests, and also with the prevailing moisture and temperature conditions of the air—which were also essentially constant in the tests here considered. In general, it seems safe to suppose that the lag in our regular experiments with short-stemmed plants was mostly due to readjustments occurring in the leaves, or perhaps in leaves and roots together, and that stem readjustments—although not entirely absent in the short stems—were of little importance in determining the length of the lag period or the volume of water absorbed during that period.

Any alteration in the root tissues that would increase resistance to the entrance of water from the surroundings through these tissues into the vessels, or any change in the liquid surrounding the roots that would increase external resistance to absorption, might be expected to increase the length of the lag period here considered, and this thought led to some tests on the influence of sudden changes in some chemical and physical characteristics of the solution about the roots of our solution cultures. A number of students (2, 11, 12, 15) have found that acids and alkalis at suitable low concentrations increased the absorption rate but that it was decreased when higher concentrations of these or other solutes were employed. MUENSCHER (11) found that transpiration was greatly retarded when a dilute nutrient solution about the roots was replaced by a sufficiently more concentrated one. REED (12) found that some organic acids and salts, when present around the roots at suitable concentrations, accelerated transpiration, whereas suitable concentrations of some organic acids retarded it.

The introduction of solutes into the liquid around plant roots may retard the absorption rate through an increase in the osmotic value of the medium or through specific physiological or chemical effects on the absorbing cells of the roots—or even on other tissues, as of stem and leaf. In the first case the solutes would act physically, by remaining outside of the roots and hindering water entrance. In the second case they would penetrate into the root tissue before producing their effect. Several possible ways might be suggested by which a chemically active substance that has already passed into the plant across the absorbing surfaces of the roots might influence water absorption through action on living cells in roots, stem, or foliage. WILSON and RUNNELS found that spraying leaves with Bordeaux mixture and various other materials (19, 20) increased the transpiration rate, and that a wide variety of chemical compounds increased the rate of desiccation of plant tissue (14). Placing various inorganic materials on the leaves produced an effect opposite to that produced when other substances were introduced into the solution around the roots, although the principles involved were perhaps similar.

In some of our experiments, the steady absorption rate of a willow plant was first ascertained, for calm air and for a 4.4-mi. wind, while the roots were in distilled water, after which the plant was transferred to another bottle filled with 0.01-M solution of HCl or KOH, or with a stronger solution of sucrose or  $\text{KNO}_3$ , and the new absorption rate was then measured as quickly as possible, first in calm air and then with wind. Different individual plants were used for the different solutions.

Absorption was markedly retarded in every instance. The retardation was computed in each case as a percentage on the basis of the corresponding absorption rate recorded just previously for the same plant when in distilled water. With 0.01-M HCl and with 0.01-M KOH absorption was very soon retarded about 14 per cent. in calm air and about 16 per cent. in the 4.4-mi. wind. This retardation cannot be regarded as due to osmotic influence of the solutions, for the osmotic values of these were very much less than the osmotic pressures commonly prevailing in the cells of healthy roots. Retardation supervened so quickly that it could hardly have been brought about through chemical effects of the solutes upon stem and leaf tissues after passage through the root cortex into the vessels. It was probably due to some chemical influence of these substances on the cortical tissue of the roots, which resulted in increasing the resistance of that tissue to penetration of water through it into the vessels, or perhaps in decreasing the activity of that tissue in driving water into the vessels—if any such activity was present. Apparently HCl and KOH (or their ions) penetrated into the cortical cells of the roots almost instantly when the root system was placed in the acid or alkaline solution, there causing rapid and considerable

lowering of the absorbing capacity of the root surfaces. It is clear that such an increase in root resistance to water entrance would lower the hydrostatic pressure throughout vessels and foliar tissues, and retardation of transpiration would be expected to result, as through incipient drying of the leaves. We wish to emphasize merely that these very weak solutions of HCl and KOH did retard absorption promptly and considerably and that their primary effect must have been chemical rather than osmotic, probably upon roots rather than upon stems or leaves. We have no evidence concerning their effect, if any, upon transpiration, but it may be presumed that water loss was retarded when root resistance to absorption was increased and absorption rate was reduced.

With 0.4-M and 0.5-M solutions of sucrose and with 0.5-M solution of  $\text{KNO}_3$ , definite values for the absorption rate could not be obtained; absorption was immediately decreased to a marked degree, but its decrease continued for many minutes, at a progressively slower rate of retardation; *i.e.*, the lag period was indefinite. Therefore the results obtained are no more than approximations. In these cases the new rate was recorded as that of the fifth 0.1 ml. removed from the potometer after the plant had been transferred from water to solution. These more concentrated solutions were surely hypertonic to the root cells (25), for they were observed to produce flaccidity (plasmolysis) in the terminal regions of the ultimate root branches.

The percentage retardations found for sucrose solutions with concentrations of 0.1, 0.2, 0.4, and 0.5 were, respectively, 38, 54, 68, and 76 with calm air, and about the same with wind—namely, 41, 58, 71, and 70. The 0.5-M concentration of  $\text{KNO}_3$ , which was the only solution of this salt tested, gave retardation percentages of 62 (calm) and 75 (wind). These percentages are similar to those observed with 0.4-M solution of sucrose.

From the results reported by many writers on plasmolysis and turgor changes induced by solutions of sucrose and of  $\text{KNO}_3$ , it seems safe to suppose that the absorption retardations produced by these substances in our tests were primarily due to osmotic rather than to chemical characteristics of the solutes in question. Our thought is that the retardations observed with the weaker sucrose solutions may represent nothing more than the effects of environmental osmotic resistance to water absorption by the plant roots, somewhat as though the roots had been brought into soils of inadequate water-supplying power. In the stronger solutions root injury was probably mainly or entirely due to hypertonic environmental osmotic pressure rather than to chemical stimulation or toxic influence.

It is clear from the percentage values just set forth that, for a given set of air conditions, absorption was slower as the sucrose solution was more concentrated, but it is equally clear that retardation of absorption was not

proportional to sucrose concentration nor to the osmotic value of the sucrose solution used. As has been noted, the full effect of the higher concentrations of sucrose was not registered, which may have been due, in greater or less degree, to slow penetration of this eminently non-toxic substance into the root cells and even beyond them.

The brief report of LIVINGSTON, HEMMI, and WILSON (8), concerning their experiments on the hydrostatic relations of soil moisture to plant growth and to the water-supplying power of the soil, suggested that a mercury barostat might be employed to maintain low hydrostatic pressures in our culture bottles, thus substituting the hydrostatic influence of a mercury column—as in LIVINGSTON'S porous-porcelain auto-irrigator (6) and in WILSON'S (18) and RICHARD'S (13) irrigator pots—for the above-mentioned osmotic influence of sucrose solutions. This suggestion proved impracticable however; with a mercury column exerting a suction of 0.1 atmosphere on the nutrient solution in the culture bottle, the rate of absorption by the plant was not appreciably reduced, and when the barostat was arranged to give a suction of 0.2 atmosphere, air found its way into the bottle, *via* lenticels of that part of the plant stem which was within the bottle, and prevented potometric measurement of the absorption rate. As has been seen, our plants absorbed water regularly from sucrose and  $\text{KNO}_3$  solutions representing osmotic pressures much greater than 0.2 atmosphere, and it seems certain that regular absorption from weak nutrient solution with greatly reduced (or even negative) hydrostatic pressure might have occurred had it been practicable to prevent air entrance into our apparatus from the plant.

These relations concerning the lag in potometer response to wind and to changes in the characteristics of solutions about the plant roots may be worthy of much more elaborate and prolonged experimental analysis.

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# HYDROGEN ION CONCENTRATION AND SEXUAL EXPRESSION IN *LYCHNIS DIOICA* L.<sup>1</sup>

J. FISHER STANFIELD

(WITH TWO FIGURES)

## Introduction

Considerable work has recently been done on the physico-chemical differences between staminate and pistillate plants of dioecious species in an attempt to formulate an objective statement of the chief sexual contrasts (4, 16, 19, 26, 27, 28). Although not purely physico-chemical in nature, closely related work stresses the genetical and morphological aspects of the problem (2, 3, 8, 20, 22, 37). Earlier physico-chemical investigations most commonly involved gross analyses either of entire plants or tops, little study having been made of separate parts or localized tissues. Hydrion studies, like other determinations of tissue fluids, have generally been limited to analysis of sap expressed from entire tops of the two sexes, a procedure which leaves much to be desired as to precision and reliability. The general usefulness of recently developed vital staining techniques and of micro-chemical tests on living tissues suggested that their application to dioecious plants might improve materially our present understanding of the physiology underlying sexual dimorphism, especially if such determinations were compared with analyses of expressed sap.

## Methods

Initial determinations reported herein involved tests on sections of living material by the range indicator method (R.I.M.) of SMALL (29) and on sap expressed from entire tops, minus buds and flowers. Only leaves were available in the rosette plants since no obvious stem had developed.

The range (5, 29) of the indicator used (fig. 1) was determined potentiometrically in well-buffered solutions. No arbitrary ranges were set. In each case the pH range given represents the definite appearance of the color which is indicative of the pH in question. This color is taken from a very thin layer of the indicator which will account for any variations from the standard ranges which are determined with a much thicker layer of liquid. Thus, for example, orange appears to be yellow when viewed in a very thin layer. Indicators in 5 per cent. alcohol were used in concentrations of 0.01 to 0.04 per cent. Free hand sections of fresh tissues were used and checked with control sections for natural coloration. The sections were left in the indicators for 8 to 10 hours. All collections were made in the morning

<sup>1</sup> Contribution no. 52 from the Biological Laboratories of Knox College.

DYE	CONC.	2	3	4	5	6	7
BROM PHENOL BLUE	.04%	YELL 3.1	3.7	BLUE			
BROM CRESOL GREEN	.04%	YELL	3.7	4.8	BLUE		
BENZENE-AZO-A-NAPHTHYLAMINE	.01%	RED	4.1	4.9	YELLOW		
ALIZARIN RED	.04%	YELLOW	4.9	5.1	PURPLE		
BROM CRESOL PURPLE	.04%	YELLOW	5.1	6.3	PURPLE		
METHYL RED	.03%	RED	5.1	5.9	YELLOW		
DI-ETHYL RED	.04%	RED	5.3	6.2	YELLOW		
BROM THYMOL BLUE	.04%	YELLOW	5.9	7.2	BLUE		

FIG. 1. Ranges and colors of indicators used in determinations of pH by the range indicator method. The critical ranges are indicated by black bars.

before nine o'clock. Examinations of the tissues were made under the low power of a microscope by means of artificial light to insure uniformity. A Corning glass globe with a clear, blue Mazda lamp of 75 watts was used as a source of light. No coverslip was used over the sections owing to the possibility of acidification. A culture slide with a coverslip may be used satisfactorily however. Since this method seeks merely to determine the approximate range of pH, only definite colors were required, these colors being the end points given (fig. 1).

Further comments on this method and its results are given in an earlier paper by the writer and others (5, 18, 24, 30, 31, 34, 35, 36). A complete and critical description of the R.I.M. has been given by SMALL (29).

In making tests potentiometrically on expressed sap all materials were frozen for several hours in dry ice in a specially constructed container. The sap was extracted by means of a hydraulic cylinder press under approximately 10,000 lb. per square inch. Determinations were made immediately after extraction. In all comparative determinations made, the plants of opposite sexual expression were grown and tested under identical conditions. *Lychnis dioica* was selected for these studies because of the marked sexual contrasts of the plant, its definite dioecism, the infrequency of sex reversals, and the abundant previous work on the cytology of sex in the genus. Physiological study supplemental to existing cyto-anatomical data is required to clarify the functional changes preceding the anatomical transitions of the two sexes. Such studies should enhance our understanding of sexual differentiation and its ultimate expression in this species in pistillate and staminate parts each with its characteristic metabolism.

The data from the various tests are presented under two main headings: (1) range indicator tests, and (2) potentiometric tests.

## Data

## RANGE INDICATOR METHOD DETERMINATIONS

Comparative pH differences by the use of the range indicator method are given (table I) for entire stems of actively blooming staminate and

TABLE I  
DIFFERENCES IN pH RANGE OF STEM TISSUES OF STAMINATE AND PISTILLATE PLANTS OF  
*LYCHNIS DIOICA* L.

TISSUE	RANGE OF pH OF PLANTS	
	PISTILLATE	STAMINATE
	<i>pH</i>	<i>pH</i>
Epidermis .....	3.8-5.2	4.8-5.2
Cortex .....	4.8-5.2	4.8-6.0
Sclerenchyma .....	3.8-5.2	4.8-5.2
Pericycle .....	4.8-5.2	4.8-6.0
Phloem .....	4.8-6.0	5.0-6.0
Xylem .....	3.8-5.2	4.8-5.2
Pith .....	4.8-5.2	5.0-6.0

pistillate plants. Tests were made on both sexes of 20 different plants grown in the greenhouse. The figures given represent the definite tendency in terms of the R.I.M. In general, the staminate tissues show a higher pH than the pistillate in practically every case. The epidermis, sclerenchyma, and xylem are always more acid than the other tissues in the stems of both sexes. The test on the phloem represents a regional reaction rather than that of a specific tissue.

Comparative data for staminate and pistillate flowers are given (table II). Photomicrographs of the flowers indicate the relationship of floral parts used for the determinations and comparisons (fig. 2).

TABLE II  
DIFFERENCES IN pH RANGE OF FLOWERS OF STAMINATE AND PISTILLATE PLANTS OF  
*LYCHNIS DIOICA* L.

PISTILLATE FLOWERS		STAMINATE FLOWERS	
FLORAL PART	RANGE OF pH	FLORAL PART	RANGE OF pH
	<i>pH</i>		<i>pH</i>
Placenta .....	4.8-5.2	Vascular strands .....	5.0-6.0
Vascular strands .....	5.0-6.0	Pseudo-receptacle .....	5.0-6.0
Ovule .....	5.0-6.0	Base of filament .....	4.8-5.2
Ovary wall .....	4.8-5.2	Base of petals .....	4.8-5.2
		Rudimentary pistil .....	5.0-6.0

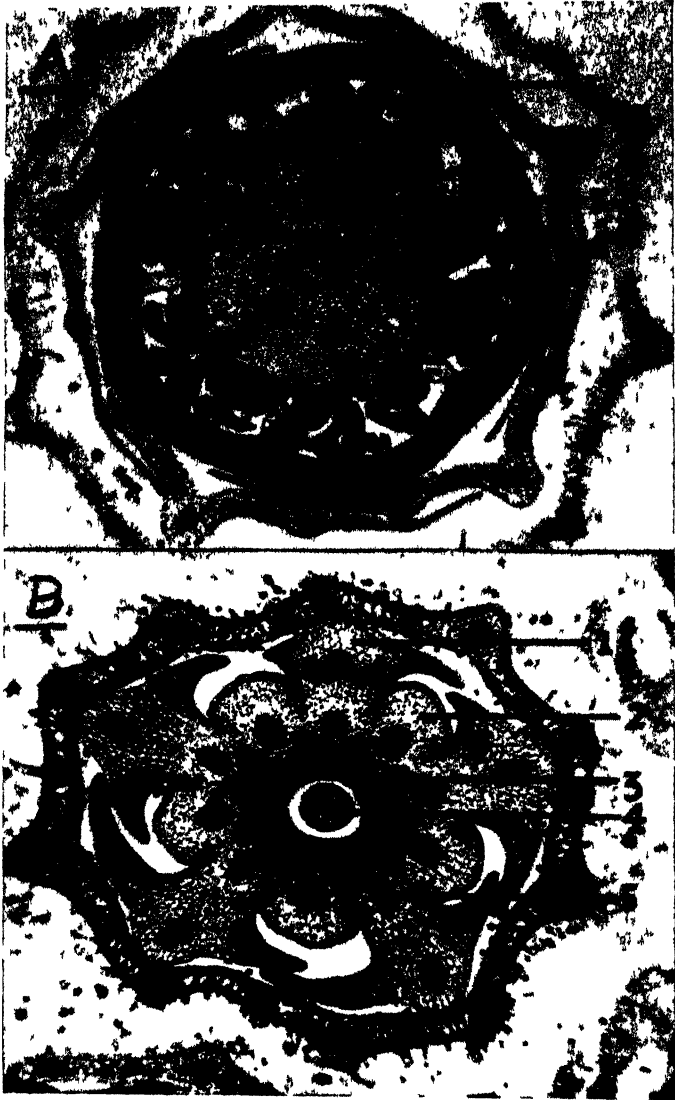


FIG. 2. Cross-sections of *Lychnis dioica* flowers. A. Cross-section of pistillate flower: 1, ovary wall; 2, ovule; 3, vascular strands; 4, placenta. B. Cross-section of staminate flower: 1, base of petal; 2, base of filament; 3, pseudo-receptacle; 4, rudimentary pistil; 5, vascular strands.

It will be noted that in the staminate flower the receptacle and its associated parts are more alkaline than either the stamen bases or the corolla lobes. The pH of the rudimentary pistil found in the male flowers (7) is the same as that of the pseudo-receptacle although differentiated from

it morphologically. Owing to difficulties in preparing material, no tests were made on the calyx. The most striking condition noted in the tests on the pistillate flower is the alkalinity of the ovules and the vascular strands as contrasted with the ovary wall and the placenta. In all tests made, this relationship remained definitely established although subject to minor variations. It was found that certain parts of the flowers of opposite sexes have the same pH range. For example, the receptacle and vascular strands of the staminate flower show the same pH range as do the ovules and the vascular strands of the pistillate flower. In like manner, the ovary wall of the pistillate flower and the base of the petals and stamen filaments of the staminate flower have identical pH ranges. The pseudo-receptacle of the staminate flower is consistently higher in its pH range than the placenta of the pistillate flower. This is of some significance since these structures occupy the major portion of the central area of each type of flower and because their mass dominates the appearance of the entire cross-section. Contrary to the condition found in the stem tissues, studies of the floral parts, as indicated above, do not show any consistent tendency toward a higher pH for either sex. Thus all differences must be expressed only in terms of individual floral parts, not in terms of the flower as an organ. Such differences in the various portions of the flower, however, are extremely consistent and therefore of diagnostic value equal to any obtained through the study of stem tissues.

#### POTENTIOMETRIC DETERMINATIONS

In these tests, all parts of the plant above-ground were used with the exception of flowers and buds (table III). *Lychnis* is a long-day plant, and

TABLE III

DIFFERENCE IN pH OF EXPRESSED SAP OF PISTILLATE AND STAMINATE PLANTS OF  
*LYCHNIS DIOICA* L.

MATERIALS TESTED	pH	
	PISTILLATE	STAMINATE
Rosette, pre-blooming stage, in greenhouse	6.8	6.8
Young plants, early blooming stage, in greenhouse	6.4	6.0
Actively blooming stage, in greenhouse	6.6	5.5
Late blooming stage, in greenhouse	6.3	5.0
Post-blooming stage, in greenhouse	6.7	6.8
Late blooming stage, in garden	5.9	5.1

it is thus possible to keep it in a vegetative, or rosette, stage indefinitely by growing it under short-day conditions where the day length is less than

13-14 hours. Such plants can be grown in all seasons under the usual greenhouse conditions of light and temperature. The plants listed as rosette plants had thus shown no sexual differentiation and were forced into bloom and the sex determined after these tests were made. This would indicate what their sex would have been if they possessed certain inherent qualities in the rosette stage which would predetermine their sexual expression when forced into bloom. Before sexual differentiation, there is no difference in the pH, and the same is true for the post-blooming stage. It is only in the blooming stage that a difference in the pH occurs. The pistillate plants are always more alkaline than the staminate in all stages of flowering and under all growth conditions. The floral parts were not included in making these tests.

There was a pronounced drop in the pH of the staminate plant upon blooming. The tests, as a whole, run somewhat higher in their pH than do the tests on similar plants with the R.I.M. An increase in acidity concomitant with the age of blooming plants is noted, particularly in the staminate plants. Young plants 10 to 14 weeks of age which have just come into bloom, as a rule, show a higher pH than do those which have been blooming for a longer period. During the blooming period, all such plants, regardless of sex, are more acid than any plants during either their pre- or post-blooming existence. Again this is more striking in the case of the males.

### Discussion

Few available data deal with the physiological properties of *Lychnis*. KEEGAN (17) worked with this plant, but none of his analyses apply in any way to the dioecious condition found in this form. No comprehensive study of the physiological aspects of sex differentiation in this plant have been found in the literature, and hence comparison is possible only with the results obtained from other plants. (SATINA and BLAKESLEE (25), in their work on the biochemical differences between sexes in green plants, found that the female plants of *Rhamnus*, *Morus*, *Ailanthus*, and *Rumex* were higher than the males in total acidity as determined roughly by titration with potassium hydroxide. pH determinations on the sap of *Rhamnus*, *Morus*, and *Cannabis* showed no significant sexual differences. HOKMEIER (13), in an unpublished work on the buffer capacity in relation to sex in dioecious plants, found that the expressed sap of mature male plants of hemp (*Cannabis sativa*) was more acid than that of the female and had a better buffer capacity. The buffering was more marked against acid than base. TALLEY (32), in a study made on the same plant, found that the males were more acid and better buffered than were the females, although the females fluctuated less in their pH. He worked only with fully matured plants. In a later work (33) he also found the active acidity of expressed

sap of staminate plants to be greater than that of female plants. LOEHWING (19) found that the tops of 15-week-old spinach plants were slightly more acid in the case of the female plants. Likewise in hemp he found that the tops of 10-week-old plants showed a slightly higher pH for the males. This difference was still more marked in plants 7 weeks of age. Such potentiometric studies show that the different sexes of some dioecious plants vary in their respective concentrations of hydrogen ions. The pH as such is no criterion of sex in general but in comparative tests on opposite sexes of the same species differences are shown.

ATKINS (1), using micro-colorimetric methods for the determination of pH values, found that the xylem was more acid than the pith and medullary rays, and that the midrib of the leaf was more acid than the parenchyma. ROGERS and SHIVE (24), using the R.I.M., noted that the pith, phloem, and cortex were more alkaline than the other tissues in the stem of *Oxalis*; the phloem, in particular, was more alkaline in the stems of *Rumex* and *Zea*. A review of the literature would indicate that SMALL and his collaborators (29), in their use of the R.I.M., have the most extensive and detailed summary of the results on the variation of the pH of plant tissues. These results all indicate differences not only in the pH of extracted sap of plant organs but also in the tissues of these organs by means of the R.I.M. It is thus to be expected that similar variations might be found in the determinations given herein, and such is the case.

The results of the present study show (table III) that in actively blooming plants of *Lychnis*, the expressed sap of the staminate plants is more acid than the sap of the pistillate plants. A similar condition with regard to the expressed sap of *Cannabis* was found to exist by HOXMEIER (13) and TALLEY (33). Both of these workers used the entire plant above-ground. LOEHWING (19), however, found that, in the case of spinach, the pistillate plants were slightly more acid than the staminate, and in hemp he found the same. Such variations in the findings might be due to factors in the environment under which the plants were grown, since methods of testing were the same. Similar methods were followed by the writer. It might also be due to the parts of the plants used for testing, and their age. These are both variable factors (6, 9, 10, 11, 14, 15, 21). More data are necessary to evaluate these studies. SATINA and BLAKESLEE (26) found no significant sexual differences in the pH of the sap of some plants studied (*Rhamnus*, *Morus*, and *Cannabis*). The above records show varying results as regards the consistency of the correlation of the pH of extracted plant saps and of their sexual expression in the various genera tested. It is to be noted, however, that in *Lychnis*, during the blooming period, the staminate plants, consistently and under all conditions of growth, have a lower pH than the pistillate plants and thus are more acid. In every case, plants compared



were grown and tested under identical conditions. On the other hand, *Lychnis* shows no difference in the pH of the extracted sap from rosette plants which have not as yet showed any sexual differentiation. The same lack of contrast holds true for the plants during the post-blooming stage. The increase in acidity of plants concomitant with their blooming is quite obvious when compared with the pre- and post-blooming stages. The actual increase in acidity in the staminate plants is much greater than that found in the pistillate plants. The male plant characteristically remains below neutral in its pH and is thus always on the acid side, according to both the potentiometric method and R.I.M.

No previous work has come to the writer's attention which treats of the R.I.M. for determining the pH of cells and tissues in *Lychnis dioica* L. except the work of SMALL (29) and REA and SMALL (23). Their determinations did not deal with possible correlations of hydrion concentration with sex in dioecious forms but were confined to tissues within the stem and flower respectively. In using the R.I.M. it is found that, in general, the stem tissues of the pistillate plants are more strongly acid than those of the staminate. In both sexes, however, it is clearly demonstrated that the epidermis, xylem, and sclerenchyma are more acid than the other tissues of the stem. This condition seems to be rather general in the stems of plants, according to ATKINS (1), SMALL (29), and ROGERS and SHIVE (24). It may result from a more acid condition of their cell walls due to lignification. The fact that the phloem and pith showed the least acid tendencies correlates well with the observations of SMALL (29) on the Caryophyllaceae, to which *Lychnis* belongs. A similar condition was found in *Oxalis*, *Rumex*, and *Zea* by ROGERS and SHIVE (24).

There are many factors to be considered in any comparison of the potentiometric method and the R.I.M. of hydrion determination. This is shown by the fact that the stems of the staminate plants have, in general, a pH higher than that of the stem of the pistillate plants with the R.I.M. Opposite results are obtained with expressed sap. That is, the major tissues of the stem of the two sexes show differences by the use of the R.I.M. that are not obvious in potentiometric determinations on the expressed sap of such a highly differentiated organ. This should not invalidate either of the results, because the ranges obtained with the R.I.M. are quite large and very nearly include the limits of the variation shown between the two sexes by the potentiometric determinations. It is probable that a refinement of the R.I.M. with smaller and more definite categories would give results more directly comparable to those obtained by the use of the potentiometric method, but a detailed discussion of the comparative worth of the two methods of testing is beyond the scope of this work. Small differences in

the pH are often difficult to distinguish by the R.I.M. due to the inability of the observer to differentiate color values in certain ranges.

Certain definite and consistent differences between the pH of tissues can however be demonstrated within the organs of plants by means of the R.I.M. The strikingly acid condition of the woody and cutinized tissues of the stem cannot be shown in expressed sap from crushed whole stem. These tissues would yield little sap in comparison with the more succulent parenchymatous tissues which normally show a higher comparative pH by the R.I.M.

Comparative studies of the floral sections by the R.I.M. indicate a more alkaline tendency in the staminate tissues. This alkalinity is conducive to an increased oxidase activity since this enzyme complex is known to function more effectively at or above the neutrality point. Determinations made by the writer on sap expressed from the floral buds of both sexes are in agreement with this fact since the staminate buds show a much greater oxidizing power than do the pistillate. The higher pH of the expressed sap of entire plants, minus buds and flowers, concomitant with femaleness in all stages of blooming, is also in agreement with this general tendency. In each of the three stages of blooming studied (table III) the pistillate plants were found to have a higher pH and to be higher in oxidase activity. (Unpublished data.) It has been further noted that the floral buds differ in a striking fashion from the vegetative parts in their oxidizing powers. The staminate plant has the higher rate of the two in the floral buds, while the pistillate plant has the higher rate in the vegetative parts. The high degree of correlation found in these tests lends further support to the correctness of the general relationship between hydrion concentration in tissues and their oxidase activity. The greater acidity of the pistillate flower, as shown by the R.I.M., appears to be due to the greater amount of oxalic acid in its major tissues, since the placenta and wall of the ovary with a low pH contain more calcium oxalate crystals than analogous parts of the staminate flower.

### Summary

#### RANGE INDICATOR DATA

1. The acidity of various tissues of the staminate and pistillate plants of *Lychnis dioica* was determined by means of the range indicator method, using a series of suitable indicators. Sections of the living stems and flowers were microscopically compared.

2. In both sexes, the xylem, epidermis, and sclerenchyma were consistently more acid than the other tissues of the stem. The phloem and pith were consistently more alkaline than the other tissues.

3. In general, the stems of the staminate plants show a higher pH than those of the pistillate, viz., a more alkaline tendency in all tissues.

4. The ovules and vascular strands connected with them are consistently more alkaline than other tissues of the ovary.
5. The ovary wall in the pistillate flower, the base of the stamen filament, and the base of the petals in the staminate flowers show a consistent similarity in the pH range.
6. The pseudo-receptacle of the staminate flower is always more acid than the placenta of the pistillate flower.
7. No general pH range can be said to differentiate staminate from pistillate flowers except by reference to individual tissues mentioned above.

#### POTENTIOMETRIC DATA

1. Comparative tests were made on the expressed sap of *Lychnis dioica* L. by means of the potentiometer. The entire plant above-ground was used, with the exception of flowers and buds.
2. In the actively blooming stage, the expressed sap of the staminate plants is consistently and under all conditions of growth more acid than that of the pistillate plants. No recognizable differences in the pH are noted in either the pre- or the post-blooming stages.
3. A general increase in acidity is noted, concomitant with blooming, in both sexes. The staminate plants become more acid than do the pistillate.
4. No correlation between any definite pH range and the expression of maleness or femaleness is to be noted in these potentiometric determinations.
5. In general, there is an increase in acidity with age in plants of both sexes during the blooming season. The younger plants are less acid.

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# NITROGEN IN RELATION TO THE GROWTH OF CITRUS CUTTINGS IN SOLUTION CULTURES

A . B . C . H A A S

(WITH FIVE FIGURES)

The commercial culture of citrus in most soils of California requires some form of nitrogen fertilization. Under certain conditions nitrogen may be readily carried to depths below the root zone. In addition, with trees of large size it is frequently difficult to maintain appreciable concentrations of nitrate in the soil solution on account of the large nitrogen requirement of the trees (5).

A healthy state of growth in citrus trees frequently is associated with an extremely low concentration of nitrate nitrogen in the soil, and a poor state of growth with a relatively high concentration. In the former case the nitrate is rapidly absorbed while in the latter it is absorbed very slowly because of high concentrations of chlorides or because of some other unfavorable condition.

It is desirable, on account of the cost, to keep the nitrate level as low as is consistent with a healthy growth response. In the effort to accomplish this it is possible that the roots during certain periods may be bathed by a soil solution containing little, if any, nitrate or other forms of nitrogen. It has been shown that the growth of citrus in solution cultures is benefited by the temporary depletion of certain elements (3). The length of such depletion period, however, and the nature of the other ions in the solution, determine in a large measure whether or not actual injury shall follow.

## Deficiency of nitrogen in solution cultures

Rooted leafy-twigg cuttings of rough lemon were grown in a culture solution of the following composition (p.p.m.):

Na	K	Ca	Mg	Cl	NO <sub>3</sub>	SO <sub>4</sub>	PO <sub>4</sub>	B	Mn	Fe
7	185	159	54	10	718	216	105	0.5	6.2	0.1

Five-gallon capacity shallow enamelware pans were used for the cultures and the solution was not otherwise aerated. It was found by testing the culture solution that nitrogen was the first element to become depleted. The culture solution<sup>1</sup> was renewed at frequent intervals and the cuttings were grown until they were about four feet high.

<sup>1</sup> In all cultures reported in this paper, phosphate was added for a short time prior to the renewal of the culture solution. When phosphate was absent, 5 p.p.m. of aluminum was added in the form of the citrate to the culture solution.

When this growth was attained, the culture solution was not renewed. After a time, the nitrate became fully depleted while a considerable amount of calcium was still present in the culture solution. This condition was soon followed by a marked response in the appearance of the cuttings.

The roots became gelatinous and bluish in color. The tops showed no immediate effect, although there was a gradual loss of leaves during the period of equilibrium restoration between the top and root. The odor of decomposing roots was strong for some distance away whenever a culture vessel cover was momentarily lifted. At this stage, a solution of calcium nitrate was added to the unchanged culture solution sufficient to increase the calcium content by 159 and the nitrate nitrogen by 493 p.p.m.

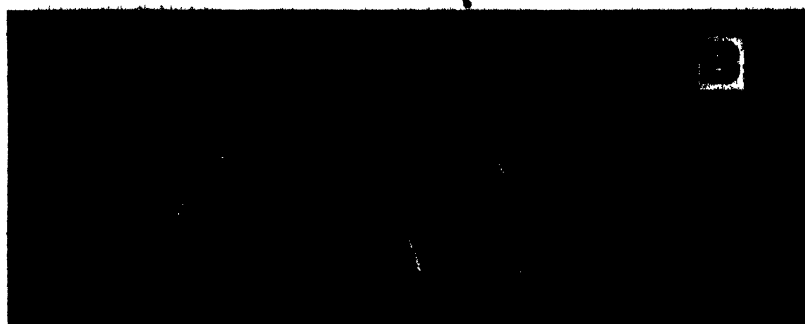
Overnight the odor of the solution disappeared, the colloidal matter in the culture solution settled, and within a few days new growth was evident in the older portion of the root system as seen in figure 1. The gelatinous disintegrating portion gradually separated from the living portion of the root system and settled.

Figure 1 A shows the darkening and collapse of the roots after the nitrate nitrogen in the solution was depleted. The effectiveness of adding calcium nitrate to the unchanged culture solution may be seen in the initial growth of white root tips from the live older portion of the root system. Figure 1 B shows a stage in the later growth of the new roots and illustrates the sloughing away of the gelatinized portions. Control cultures developed excellent roots that did not undergo these changes. In these control cultures the renewal of the solutions was not interrupted except in a few cultures in which the occurrence of a period of nitrate deficiency was avoided by the addition of calcium nitrate to the unchanged culture solution. It should be stated, although the causes are not understood, that the growth of citrus cuttings is considerably better in solutions that are frequently changed than in solutions less frequently renewed but in which attempts are made to maintain given concentrations of certain elements. Causes for such a difference may be found to be associated with the concentrations of the so-called minor elements or of toxins in the solution.

The effect of a nitrate-nitrogen deficiency on the roots of leafy-twigg cuttings of Lisbon lemon grown in culture solution is seen in figure 1 C. The cuttings were grown for a year without a period of nitrogen deficiency

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FIG. 1. Nitrogen-deficiency and recovery effects in the roots of citrus cuttings grown in solution cultures. A, gelatinization of rough lemon roots in the absence of nitrogen and the initial stage of new root growth following the addition of calcium nitrate to the unchanged nitrogen-depleted culture solution. B, a later stage in the growth of the new roots which is accompanied by the sloughing away of gelatinized roots. C, similar effects on roots of cuttings of Lisbon lemon in which the period of nitrogen deficiency was not prolonged.





because of the frequent renewal of the culture solution. Nitrate was then allowed to become deficient in the manner previously described. The period of nitrogen deficiency was not unduly prolonged before calcium nitrate was added to the unchanged culture solution. New root growth from the living or least injured portion of the root system (fig. 1 C) indicated the beginning of the recovery process.

Cuttings of Valencia orange as scion, on cuttings of rough lemon as stock, were grown for a year in a culture solution containing nitrate nitrogen, and then were grown in a culture solution containing no nitrogen. The composition of the nitrate-free culture solution was as follows (p.p.m.):

Na	K	Ca	Mg	Cl	NO <sub>3</sub>	SO <sub>4</sub>	PO <sub>4</sub>	B	Mn	Fe
7	185	159	54	280	0	216	105	0.5	6.2	0.1

The deficiency was allowed to continue until the growth of the roots was definitely at a standstill before calcium nitrate was added to the solution. The addition of nitrate prevented the collapse of the roots. The deficiency of nitrogen, though not for a period of sufficient duration to destroy the root system, nevertheless severely injured it. The youngest roots failed to respond to the addition of the nitrate. The growth response in the root system during the following year was, therefore, entirely from the older, less injured roots.

It was found that small, freshly-rooted cuttings of rough lemon were able to withstand a nitrogen deficiency for a long time when placed directly into the nitrate-free culture solution. The new top growth, if any, was uniformly pale green in color. When cuttings were grown to a considerable size in culture solutions containing nitrate nitrogen and were then grown for a sufficient time in a nitrogen-deficient solution, the roots were injured and the leaves reflected the root condition. The leaves in such cases became pale green, and the veins light colored (fig. 2 A) which usually signifies that the root system is injured. This light-colored vein condition is readily distinguishable from the effects known as chlorosis (fig. 2 B) in citrus leaves in which the veins are green while the remainder of the blade assumes a yellowish green color. Chlorosis may be brought about in many ways, chief among which is the factor of pH.

### Nitrogen effects on the growth of cuttings

Previous investigations (HAAS, 2) have shown that certain forms of nitrogen, when continuously supplied to sand or soil cultures in amounts that insure the absorption of the nitrogen in an unchanged condition, are injurious to citrus, and produce leaf symptoms as evidence of such injury. Urea, cyanamid, dicyanamid, and closely related forms of nitrogen behave in this way. In view of the fact that little, if any, knowledge exists in



FIG. 2. Nitrogen-deficiency effects compared with those of chlorosis in citrus leaves. A, leaf from a cutting of Valencia orange as scion on a cutting of rough lemon as stock. The cutting was grown for a year in a culture solution containing nitrate nitrogen and then in a similar solution lacking nitrogen. B, leaf from a cutting of Valencia orange affected with chlorosis as a result of a high pH value in a culture solution containing nitrate. Note the reverse condition of the veins in A and B. C, leaf from a cutting of Valencia orange grown in a control culture solution.

regard to the effect of the form and concentration of nitrogen on the growth of citrus under controlled conditions, preliminary experiments were made in solution cultures as a basis for future investigations.

#### AMMONIA NITROGEN

Rooted leafy-twig cuttings of Lisbon lemon were grown from September 19, 1934, to April 25, 1935, in 5-gallon capacity shallow enamelware pans. The culture solution used had the following composition (p.p.m.) :

Na	K	Ca	Mg	Cl	SO <sub>4</sub>	PO <sub>4</sub>	B	Mn	Fe
3.5	71	159	27	275	156	105	0.5	6.2	0.1

The alternation of phosphate with aluminum citrate was carried on as previously mentioned. Ammonium sulphate solution was added to the culture solution in order to secure the following concentrations of nitrogen:

0, 1, 2, 3, 5, and 10 p.p.m. The culture solutions were renewed every few days and the pH was maintained at 6 to 6.5 by means of a solution of calcium hydroxide.

Growth of the tops was increasingly better as the nitrogen content of the solution increased (fig. 3). The root system was somewhat larger at



FIG. 3. Growth of leafy-twig cuttings of Lisbon lemon in solution cultures to which various concentrations (p.p.m.) of nitrogen were added in the form of ammonium sulphate.

the lower than at the higher nitrogen concentrations. In the low nitrogen cultures, some of the leaves showed slight evidence of sulphate excess (HAAS and THOMAS, 4). This is difficult to avoid because of the low nitrogen-sulphate ratio. Furthermore the absorption of ammonia in the absence of nitrate may not be entirely without injurious effects. In fact, the citrus leaves were yellowish-green in color regardless of the iron supply. The cuttings had the appearance of being in need of nitrogen. These effects of

ammonium absorption agree with those obtained in soil cultures that were given daily applications of dilute ammonia. In soil, the discontinuance of the ammonia treatment was followed by recovery as the nitrification of the ammonia progressed.

In the present solution cultures, nitrate was not found in the solution at any time, and unless the ammonia was changed to the nitrate form as it was absorbed, the only conclusion possible is that these cultures were grown with enforced ammonia absorption. It is possible, therefore, that a given concentration of ammonium becomes less injurious as the solution or the plant contains increasing amounts of nitrate. Further investigation will be necessary to secure data on the ammonia-nitrate relationship.

#### NITRATE NITROGEN

Other cuttings of Lisbon lemon were grown in a culture solution similar to that used for ammonia nitrogen. Nitrogen in the form of potassium nitrate was added to the culture solution in order to obtain the following concentrations (p.p.m.): 0, 1, 2, 3, 5, 10, and 15. Figure 4 shows the resulting growth: that at 3 or 5 p.p.m. was greater than that at 1 and 2 p.p.m. and less than that at higher concentrations. It is doubtful, therefore, whether rapidly growing cuttings, such as lemon, can secure adequate supplies of nitrogen from solutions containing extremely low concentrations of this constituent. In the field, such low concentrations of nitrogen frequently are associated with a healthy vigorous growth in citrus but at some time prior during the year the nitrogen level in the soil far exceeded the low nitrogen concentrations.

#### NITRITE NITROGEN

Nitrites have been reported (1) as occurring in considerable concentration in certain soils. The chemical determination of nitrites in soils and plants is a difficult one because of possible changes in the form of the nitrogen that may occur under certain conditions. There has long been a suspicion that nitrite in the field at times may reach concentrations injurious to citrus, but proof of this contention is lacking. Experiments in which solution cultures were used, furnish data regarding the effect of nitrites on citrus.

Rooted leafy-twigs cuttings of Lisbon lemon were grown from July 23, 1934, to March 22, 1935, in solution cultures in 5-gallon capacity shallow enamelware pans. The culture solution contained (p.p.m.):

Na	K	Ca	Mg	Cl	NO <sub>3</sub>	SO <sub>4</sub>	PO <sub>4</sub>	B	Mn	Fe
7	185	159	54	10	785	216	105	0.5	6.2	0.1

The aluminum-phosphate relation was maintained as previously. A solution of potassium nitrite was added to the culture solutions in order to secure

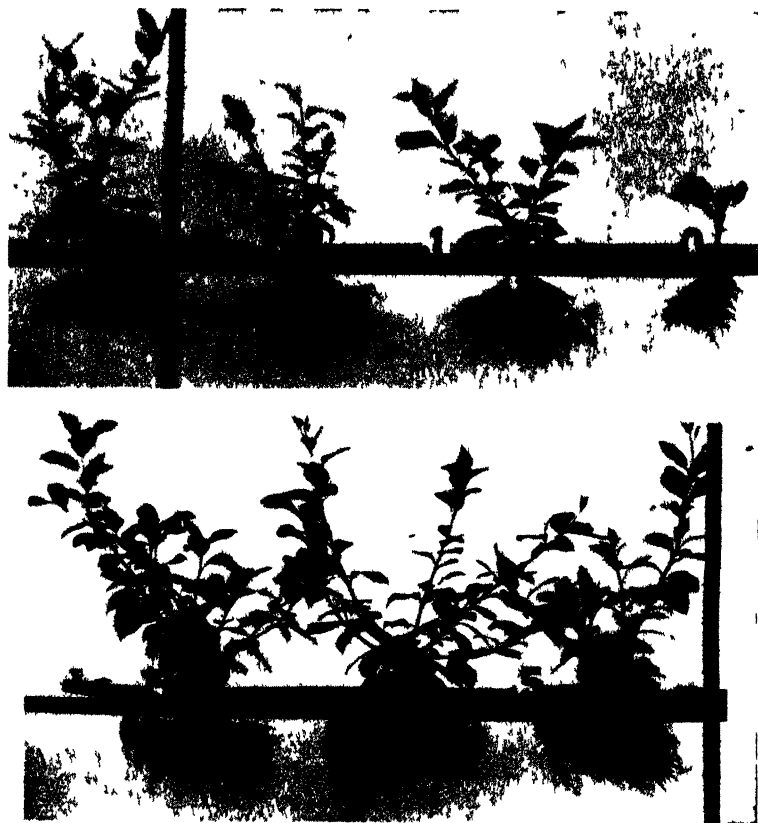


FIG. 4. Growth of leafy twig cuttings of Lisbon lemon in solution cultures containing various concentrations (p.p.m.) of nitrogen added in the form of potassium nitrate.

the following concentrations of nitrite (p.p.m.): 0, 5, 10, 15, and 25. The solutions were renewed every few days. The nitrite concentrations were maintained by testing the solutions and adding nitrite as required. The rapidity of change of nitrite to nitrate was retarded by maintaining the pH of the culture solutions in the range 7 to 7.5 by means of a solution of calcium hydroxide. Even so, the nitrite content of the solutions changed considerably within the course of a few days.

Figure 5 A shows the growth made by the cuttings in solutions containing various concentrations of nitrite. The control cultures were not distinguishable from the cultures that received 5 p.p.m. nitrite and hence are not shown in the figure. It is significant that the presence of 785 p.p.m. of nitrate has not prevented the nitrite from seriously interfering with the growth of lemon cuttings. It is not known whether the injury would have been greater in the presence of a smaller concentration of nitrate.



FIG. 5. Effect of various concentrations (p.p.m.) of nitrite on the growth of cuttings of citrus in solution cultures. A, Lisbon lemon, B, Valencia orange.

In a manner similar to the procedure just described for Lisbon lemon, rooted leafy-twigs of Valencia orange were grown from October 26, 1934, to April 25, 1935, in solutions containing nitrite. As shown in figure 5 B the growth of the roots was interfered with when the concentration of nitrite exceeded 5 p.p.m. The cuttings in the control cultures were not different in appearance from those grown with 5 p.p.m. nitrite. Above 5 p.p.m. nitrite, the finely divided roots were usually dark in color and finally became somewhat gelatinous. Recovery was rapid when nitrite additions were withheld. The injury brought about by the presence of nitrite is not characterized by symptoms that are more or less specific, hence the diagnosis and the appraisal in the field of the injury for which nitrite is responsible are at present obscure.

### Summary

1. A preliminary study was made of the growth of citrus cuttings in solution cultures with various forms and concentrations of nitrogen. Such

a study becomes of importance because the commercial culture of citrus in most soils of California requires some form of nitrogen fertilization.

2. Rooted leafy-twig cuttings of rough lemon or Lisbon lemon were grown in a so-called complete culture solution until the cuttings were several feet high. Depletion of the supply of nitrogen was then followed by a collapse of the root system accompanied by a corresponding loss of affected leaves. The addition of calcium nitrate to the nitrogen-depleted cultures brought about a recovery that varied with the degree of injury. A distinction is shown to occur between the appearance of citrus leaves in need of nitrogen and that due to chlorosis as a consequence of pH.

3. The tops of cuttings of Lisbon lemon grown in solution cultures with ammonium as the only source of nitrogen were larger with increasing concentrations of nitrogen. The leaves had the appearance of being in need of nitrogen.

4. The growth of cuttings of Lisbon lemon was greater with increasing concentrations of nitrate as the source of nitrogen in the culture solution.

5. It is doubtful whether rapidly growing cuttings of citrus can secure adequate supplies of nitrogen from solutions containing extremely low concentrations of this constituent.

6. Concentrations of nitrite above 5 p.p.m. in culture solutions that contain 785 p.p.m. of nitrate, were distinctly injurious to the growth of cuttings of Lisbon lemon and Valencia orange.

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# EFFECTS OF PHOSPHORUS AND LIME IN REDUCING ALUMINUM TOXICITY OF ACID SOILS<sup>1</sup>

KENNETH E. WRIGHT

## Introduction

There is a wide divergence of opinion as to the cause of the toxic effects of acid soils upon the growth of certain species of plants. By some this toxicity is attributed to acidity *per se*. The majority of investigators believe this toxicity is due primarily to the presence of soluble aluminum salts in the soil. Various investigators have shown that this toxic effect of acid soils can be overcome by the application of lime and of superphosphate in rather large amounts. The beneficial effects of these treatments have been variously attributed: (1) to supplying essential elements that are deficient in the plant; (2) to changing the soil reaction to a pH range that is favorable for plant growth; (3) to rendering aluminum inactive because of precipitation in the soil; and (4) to eliminating the toxic action of aluminum by supplying phosphorus in sufficient amounts both to precipitate aluminum within the plant tissues and, in addition, provide sufficient quantities for normal plant metabolism. This work was undertaken in an attempt to clarify the conflicting viewpoints just listed.

## Soil culture experiments with beets

It was desirable to find some calcium salt that could be added to an acid soil without appreciably changing its pH and yet supply calcium to the plant. Thus the rôle of calcium as an essential element could be compared with its rôle in reducing acidity. Samples of an acid soil were variously treated with different amounts of calcium lactate and calcium acetate alone and in combination with different amounts of calcium, magnesium, and sodium carbonates. As a result of this experiment it was decided that the application of calcium lactate would be the most suitable for use in the reduction of toxicity as it did not appreciably alter the pH of the acid soil and yet its application resulted in plants that showed a better development than those grown in the untreated acid soil. In addition to the calcium lactate treatment acid soil was treated with lime and with superphosphate. Beets grown in untreated acid soil were used as a check.

Determinations of calcium, magnesium, phosphorus, and total nitrogen in the plant and nitrates in the soil were made according to the standard methods as described by the ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS (1). Plant nitrates were determined according to the method of FEEAR

<sup>1</sup> Published as contribution no. 494 of the Rhode Island Agricultural Experiment Station, Kingston, R. I., by permission of the Director of Research.



(3) and total sugars and reducing sugars by the method described by SCHLENKER (7). The hydrogen ion concentration was determined by the electrometric method using a quinhydrone electrode with a saturated calomel half-cell. The results of the various analyses are shown in table I.

It is evident that pH as such is not a limiting factor in growth as the superphosphate treatment resulted in a vigorous growth of plants in contrast to the very poor development of plants grown on the acid soil, although the difference in soil pH of these two treatments was negligible. Furthermore, the calcium lactate treatment resulted in plants which showed a better general development than those of the acid soil without an appreciable change in pH.

A high calcium content of the plants grown in the acid soil indicates that a deficiency of calcium as an essential element cannot be considered as a limiting factor in growth. This is further supported by the fact that those plants grown with the calcium lactate treatment contained more calcium than the plants grown with the lime treatment, the former, however, being comparatively retarded in development.

TABLE I  
ANALYTICAL RESULTS OF SOIL CULTURE EXPERIMENTS WITH BEETS

SOIL TREATMENTS	CHECK	LIME	CALCIUM LACTATE	SUPER- PHOSPHATE
Ca in dry matter (%) .....	1.42	1.41	1.59	2.08
Mg in dry matter (%) .....	0.93	0.92	1.28	1.40
P in dry matter (%) .....	0.24	0.61	0.50	0.68
NO <sub>3</sub> in dry matter (p. p. m.) .....	264	1080	352	1140
Total N in dry matter (%) .....	2.98	3.85	2.87	3.35
Reducing sugars in dry matter (%) ..	0.86	0.60	0.38	0.79
Total sugars in dry matter (%) .....	1.54	1.09	1.20	1.22
Reducing sugars—juice (%) .....	1.35	0.33	0.30	0.21
Total sugars—juice (%) .....	1.48	0.36	0.34	0.29
NO <sub>3</sub> in soil (p. p. m.) .....				
7th week .....	48	40	22	56
8th “ .....	60	28	14	60
pH of soil .....	4.32	6.38	4.47	4.49

The analyses indicate that photosynthesis apparently was not retarded because of a lack of magnesium, as the magnesium content of the plants grown in acid soil compares favorably with that of the limed flat. Moreover, there is an accumulation of both total and reducing sugars in the poorly developed plants grown in the acid soil, indicating that the carbohydrates manufactured are not being utilized by the plant. A deficiency of total nitrogen, of nitrates, and of phosphorus in the plants from the acid soil reveals possible causes of retarded growth on acid soil.

### **Drip culture experiments with beets**

In order to determine the effects of nitrate deficiency, phosphorus deficiency, and the presence of aluminum upon the growth of beets three series of drip culture experiments were conducted. The three series of standard culture solutions used consisted of one series in which nitrates varied from 0 to 108 p. p. m.; of a second series in which phosphorus varied from 0 to 10 p. p. m.; and of a third series in which aluminum varied from 0 to 12 p. p. m. Nitrate-deficient plants before death turned yellowish and then brown. The retarded plants in both the aluminum and the phosphorus-deficient series became a solid dark purple-red in color and quite brittle in texture. The symptoms were identical with those produced in the beets grown in the untreated acid soil. These solution culture experiments indicate that the lack of phosphorus in a form available for plant utilization is the chief causal factor for the limited growth of beets grown in the previously discussed soil culture experiments, and that there is a connection between a lack of phosphorus and the presence of aluminum.

### **Drip culture experiments with barley**

In order to determine whether phosphorus is precipitated in the soil by aluminum or whether phosphorus is precipitated within the plant tissues, a type of experiment was devised in which a plant could have access to both aluminum and phosphorus but in which these two elements would not be in contact in the culture solution. This would prohibit their mutual precipitation in the culture solution. It was decided to use barley for this experiment as its fibrous root system could be separated into two halves each of which could be immersed in a different culture solution. This would not be possible in growing beets because of the tap root system.

Preliminary drip culture experiments with barley showed that 5 p. p. m. of aluminum in HARTWELL and PEMBER'S (4) culture solution was sufficiently toxic to result in a considerable reduction in yield. When barley grown in the standard culture solution lacking phosphorus was compared with that grown in the presence of aluminum a decided similarity in symptoms was evident. In both cases the roots were dwarfed, thick, and showed brownish discolorations. Under both conditions top growth was about equally reduced and a reddish discoloration appeared at the base of the stem.

In the experiment in which the root system was separated into two portions, barley seedlings one week old were planted on the contacting edge of two adjacent containers, one-half the root system being placed in sand in each container. Each half of the root system in its container received a different culture solution by a constant drip from a reservoir. By thus dividing the root system it was possible, wherever desired, for the plants to have access to both aluminum and phosphorus in separate containers. Keeping these

two elements separate prevented aluminum from precipitating the phosphorus in the culture solution.

The various culture solution modifications used are shown in table II.

At the end of an eight weeks' growing period the plants were harvested in three fractions, consisting of the tops and each half of the root system,

TABLE II

COMPARISON OF PHOSPHORUS AND ALUMINUM CONTENT OF BARLEY GROWN IN VARIOUS CULTURE SOLUTIONS

TREATMENT	PLANT FRACTION ANALYZED	YIELD DRY WEIGHT BASIS OF NO. 2 AS 100	PHOSPHORUS (DRY MATTER)	ALUMINUM (DRY MATTER)
		%	%	%
No. 1 Standard culture solution containing 5 p. p. m. of P (same treatment both sides)	Roots { left right total Tops Plant, total ..	9 10 19 75 94	0.384 0.300 0.339 0.224 0.248	0.069 0.078 0.074 0.021 0.032
No. 2 Standard culture solution containing 2.5 p. p. m. of P (same treatment both sides)	Roots { left right total Tops Plant, total ..	11 9 20 80 100	0.280 0.290 0.284 0.206 0.222	0.072 0.071 0.072 0.022 0.031
No. 3 Left: $H_3PO_4$ 5 p. p. m. of P Right: 5 p. p. m. of Al in standard culture solution lacking P	Roots { left right total Tops Plant, total ..	3 2 5 13 18	0.253 0.257 0.255 0.208 0.221	0.296 0.174 0.247 0.022 0.085
No. 4 Left: Standard culture solution containing 5 p. p. m. of P Right: 5 p. p. m. of Al in standard culture solution lacking P	Roots { left right total Tops Plant, total ..	17 8 25 65 90	0.294 0.244 0.278 0.237 0.247	0.093 0.251 0.144 0.019 0.054
No. 5 5 p. p. m. of Al in standard culture solution containing 5 p. p. m. of P (same treatment both sides)	Roots { left right total Tops Plant, total ..	7 7 14 31 45	0.230 0.243 0.237 0.222 0.227	0.142 0.263 0.203 0.024 0.079

for each treatment used. The yield for each fraction was determined, as well as the amounts of aluminum and phosphorus present. Aluminum was determined by the colorimetric method of WINTER, THRUN, and BIRD (9), and phosphorus by the colorimetric method of TRUOG and MEYER (8). The results of the analyses are recorded in table II.

It is evident that the damage to the plant as a whole is the result of the poor root system caused by the presence of aluminum. In series 3, 4, and 5, where the roots were in contact with aluminum in the culture solution, there is a comparatively large amount of aluminum in the roots. In all cases these roots show the typical symptoms that have been associated with aluminum toxicity. This localization of injury in the roots in immediate contact with aluminum is further indicated by the fact that the tops of the plants from all series show very little difference in aluminum content whether aluminum was added to the culture solution or not.

In order to facilitate the discussion a ratio of the total percentage of aluminum in the roots to the total percentage of aluminum in the tops in comparison with the yields can be used. A direct comparison of the aluminum content of the roots with total yield could be made since, considering the plant as a whole, the great variation in aluminum content of the plants is found in the roots, the aluminum content of the tops being about the same in all cases. Such a comparison would serve to emphasize the fact that the primary effects of aluminum on the plant seem to be localized in the roots with a consequent poor development of the whole plant. However, since the aluminum content of the tops does vary slightly and in view of the fact that small concentrations of aluminum are known to affect the plants experimented with, the ratio method was used. Such a comparison on the basis of ratios is as follows:

SERIES	RATIO OF $\frac{\text{ALUMINUM IN ROOTS}}{\text{ALUMINUM IN TOPS}}$	YIELD
1 .....	3.5	94
2 .....	3.3	100
3 .....	11.2	18
4 ....	7.6	90
5 .....	8.5	45

The reduction in yield in series 4 is decidedly not what might be expected by comparing the ratio of this series with that of series 5. Although the difference between these two ratios is less than unity, the yield in series 4 is twice that of series 5. A comparison of the ratio of series 4 with the ratios of series 1 and 2 shows that that of series 4 is more than twice the magnitude of either series 1 or 2. The reduction in yield in series 4, however, is not

significant. Some explanation for the good growth in series 4 is evidently necessary.

In series 4 there is no opportunity for aluminum to precipitate phosphorus on the outside of the plant since these two elements are in separate culture solutions. In comparing the analyses of the tops and the two sides of the root system it is found that most of the aluminum is in the half of the root system in direct contact with aluminum. This large amount of aluminum in one half of the root system and the low amount in the other half is directly reflected in yields. Those roots not in contact with aluminum have a yield twice as great as those in contact with aluminum. This low yield of roots on the aluminum side can be attributed only to a precipitation within the root tissues as there was no phosphorus in this culture solution. This immediate precipitation of aluminum precludes the translocation of aluminum to other parts of the plant. Consequently the side of the root system not in contact with aluminum functions almost normally and the total plant yield in series 4 is not greatly reduced over that in series 1 and 2.

Although the total phosphorus percentage in series 4 is as high as series 1 and higher than series 2, there is a reduction in yield. This again indicates precipitation of phosphorus within the plant because a high phosphorus content in association with lower yields indicates that some of this phosphorus is unavailable. This is in line with the observations of BURGESS and PEMBER (2), who treated acid soils with a heavy application of acid phosphate and found that plants grew well in this soil, although they contained about the same percentages of aluminum as the plants growing in an untreated acid soil. However, the former contained from three to five times as much phosphorus. This leads them to conclude "... that acid phosphate renders soluble aluminum salts non-toxic largely by counteracting their evil effects within the plants themselves after they have been absorbed."

Yields from series 3 were too low to permit accurate analyses, hence this series is not emphasized in reaching any definite conclusions. The low total yield is probably due to the very low yield of roots, so low that the plant probably did not obtain elements essential for its growth from the incomplete culture solutions used. The high aluminum content on that side of the root system in contact with phosphoric acid is difficult to explain, unless it had its source as a contamination in the sodium hydroxide used to adjust the pH of the phosphoric acid. However, a discussion of the results of this series was not omitted as this particular technique may be improved by subsequent investigators.

The results in series 5 may be differently interpreted but it seems quite logical to conclude that these results also support the contention that, in addition to the external precipitation, aluminum renders phosphorus unavailable internally. Although the total percentage of phosphorus in series 5 is

slightly greater than that of series 2 the actual amount of phosphorus present is much less. For example, a yield of 45 gm. dry weight with 0.227 per cent. of phosphorus, as in series 5, makes the actual amount of phosphorus present 0.102 gm. In series 2, having a yield of 100 gm. dry weight and 0.222 per cent. of phosphorus, the actual amount of phosphorus present is 0.222 gm. It could be argued that the plants in series 5 grew as much as the actual limited amount of phosphorus in the plant permitted. This leads to the conclusion that phosphorus was prevented from entering the plant in amounts sufficient to support growth by its precipitation outside the plant by aluminum. This contention might further be supported by showing that the plants in series 5 have a smaller total percentage and a smaller actual amount of phosphorus in them than series 1, although the amount of phosphorus in the culture solution was the same in both cases. This line of reasoning overlooks the consideration that if phosphorus were precipitated in the culture solution by aluminum, thus cutting down its absorption, it would follow that aluminum also would be in a form that would not readily pass through the plant membranes. The analyses, however, indicate that the roots had absorbed appreciable amounts of aluminum. In fact, the actual amounts of aluminum in the root system of series 5 are in the same range of magnitude as the amount of aluminum in the right side of the root system in series 4, where it would be impossible for precipitation to occur in the culture solution, as, in this latter instance, aluminum, but no phosphorus, was present in the culture solution. It seems logical to conclude from these results that, in addition to external precipitation, aluminum causes injury, even in this series, by precipitating phosphorus within the tissues of the plant where it causes a phosphorus deficiency which is reflected in the poor growth of the barley.

Most workers are of the opinion that superphosphate renders aluminum non-toxic because of its precipitation in the acid soil or in the culture solution. BURGESS and PEMBER (2), McGEORGE (5), and PIERRE and STUART (6) have advanced the opinion that the chief remedial action of superphosphate takes place within the plant where it renders aluminum inactive by precipitation.

The experiments, as recorded in this paper, substantiate the viewpoint that a large application of superphosphate to acid soils overcomes the toxic effects of aluminum by precipitating the aluminum within the tissues of the plant. It is, of course, admitted that the above statement is an inference as far as plants growing under field conditions are concerned. In the field it would be too much of an assumption to attribute the lessening of aluminum toxicity by superphosphate application entirely to internal mutual precipitation. It was because of external precipitation that the divided-root method in the culture solution experiments was used.

Not denying that the beneficial action of superphosphate to plants in the field may be due to some external precipitation of aluminum, it is felt that the majority of workers have overlooked the possibilities of applications of superphosphate such as may supply phosphorus in amounts sufficient to precipitate aluminum internally, and, over and above this, supply sufficient phosphorus for the metabolic processes of the plant.

### Summary

1. Beets were grown in flats in an acid soil, and in acid soil separately treated with calcium lactate, superphosphate, and lime. Healthy vigorous plants were obtained with lime and superphosphate and very poor plants in the untreated soil while those with calcium lactate were about midway in their development between these two extremes.

2. Various analyses of the above plants indicate that: (1) pH, as such, is not a limiting factor, as the pH of the acid soil, calcium lactate, and superphosphate flats was about the same; (2) a deficiency of calcium as an essential element cannot be considered as a limiting factor in growth, as all plants contained as much or more calcium than those grown in the limed flat; (3) photosynthesis cannot be considered a limiting factor, as no magnesium deficiency was discovered, and, furthermore, there was an accumulation of both total and reducing sugars in the plants grown in the acid soil; and (4) deficiencies of total nitrogen, of nitrates, and of phosphorus in the plants from the acid soil reveal possible causes of retarded growth in acid soil.

3. Drip culture experiments with beets indicated a phosphorus deficiency as the possible cause of retarded growth and that the presence of aluminum was the probable cause of this deficiency.

4. In order to determine whether aluminum caused a deficiency of phosphorus by precipitation within the tissues of the plant or externally, barley was grown in drip culture solutions wherein the root system was divided, each half being placed in separate containers and receiving different culture solutions. By this method a plant could have access to both aluminum and phosphorus, and precipitation in the culture solution could be avoided.

5. For analysis, the plants from each culture series were fractionated into three parts consisting of each half of the root system and the tops. Determinations for aluminum and for phosphorus were made on each fraction. These figures in conjunction with the yield of the plants as recorded in table II indicate that: (1) damage to the plant as a whole is probably the result of the poor root system caused by the presence of aluminum; (2) aluminum is precipitated immediately in that half of the root system in direct contact with aluminum, whereas the other half of the root system not in contact with aluminum, and the tops, grow practically as well as the check plants; and (3) in that series where each half of the root system is in a culture solution con-

taining both aluminum and phosphorus, internal precipitation evidently plays an important part in retarding growth, since, if the precipitation were external, the fairly large amounts of aluminum and phosphorus found inside the roots of these plants could not be satisfactorily explained.

6. Although the results obtained from these culture solution experiments cannot be used for plants growing under field conditions, it is felt that most workers have over-emphasized the external mutual precipitation of aluminum and phosphorus and have overlooked the fact that the internal precipitation of phosphorus by aluminum evidently plays an important rôle in the poor development of certain plants grown on acid soil. The corrective action of large applications of superphosphate to acid soils is attributed largely to the internal precipitation of aluminum by phosphorus with sufficient phosphorus remaining for the metabolic processes of the plant.

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## SEASONAL FLUCTUATIONS IN GROWTH RATES OF EXCISED TOMATO ROOT TIPS

PHILIP R. WHITE

(WITH FOUR FIGURES)

Previous to 1934 ROBBINS (10, 11), ROBBINS and MANEVAL (12, 13), KOTTE (6, 7), CHAMBERS (1, 2), MALYSHEV (8, 9), GAUTHIERET (4, 5), and WHITE (14-17) had reported the cultivation of excised root-tips of various species of plants over periods in some cases as great as 5 months. In such of these studies as extended over considerable periods of time, the growth rates showed a more or less continuous diminution, ending in loss of all cultures. Fluctuations in growth rates were apparently random in nature and of no demonstrable significance. In 1934 WHITE (18) reported experiments in which two clones of tomato root-tips were successfully grown for one year. Aside from fluctuations due to known variations in environmental factors, the cultures showed not a diminution but an *increase* in growth rate. The improvement in result is probably attributable to a more satisfactory nutrient and technique. The observed increase in growth rate was tentatively attributed to adaptation of the cultured organs to their artificial environment, and it was presumed that the growth rate would ultimately reach a constant level. The present paper proposes to report the behavior of one of these clones (clone C, 18) over an additional two years.

Since a standard procedure had not yet been developed at the time the cultures were started, the first 10 passages were of irregular length and were maintained under a variety of experimental conditions. Passages 11-23 were poor for reasons discussed elsewhere (18). With the 11th passage a standard procedure (18) was adopted which has since remained essentially unchanged. The present paper, then, records the behavior of this clone through 177 passages. Of these, 167 have had a duration of one week each and have been maintained under as uniform conditions as were available in an ordinary laboratory room. In passages 11-52, 100 roots were grown in each passage so that each growth rate is the mean of 100 measurements. In all passages subsequent to 52 this number was reduced to 25.

Figure 1 shows the growth rates of this clone of cultures during 177 passages. The records for the last 125 weeks have not been previously published. The line AA' represents the average growth level for the entire 177 weeks, 6.2 mm. per culture per day. The measured total increment recorded, exclusive of branches, is about 30,000 times the magnitude of the tissue fragment with which the clone was started. The clone is being maintained and, after 3 years' cultivation *in vitro*, is still in excellent condition.

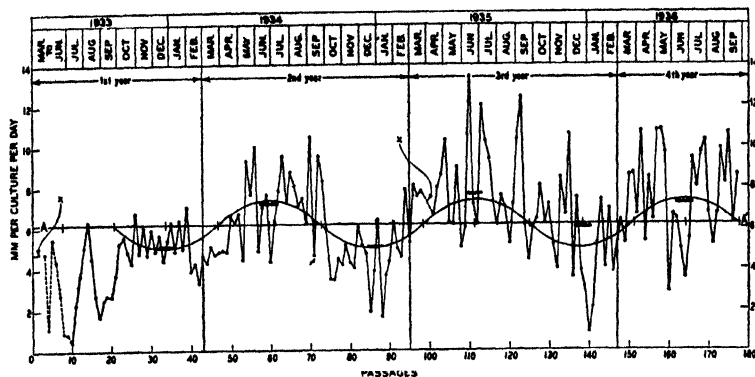


FIG. 1. Increment rates of isolated tomato root-tips over a period of 3½ years. The average weekly increments are represented by the zigzag line. The line AA' represents the average increment level for the entire period. The heavy lines at values of the sine curve  $\theta = +1$  and  $\theta = -1$  represent the average increment rates of each 26-week period beginning on September 21 and March 21, each period corresponding to a half-wave of the curve. Measurements were not recorded for passages 1, 2, 99, and 100 (marked "x").

In figure 1, the observed fluctuations in growth rate are rather uniformly grouped around the mean level AA'. The clone has, therefore, after the first period of adaptation, settled down to a uniform growth level, as was anticipated. Nevertheless, analysis of the growth curve shows it to contain two types of deviation from the mean. Random deviation from week to week is the most evident component. It seems probable that inequalities in the amount of trauma, inflicted in the manipulations of excision and transfer, may affect the subsequent growth rates. It has also been noted that the position of the explant in the parent culture—whether taken from the tip, from a branch near the center of the culture, or from the base—may affect its subsequent growth rate. There are also marked differences in the vitality of individual branches at the same level, which may be evident in the color of the meristematic region. Doubtless there are other differences which are not visible. These, and probably other factors contribute to the variability of the cultures. The range of this deviation can be greatly reduced by the use of large numbers of cultures, as is evident in passages 27 to 39 where 100 cultures were maintained. It might conceivably be eliminated by the use of sufficiently large numbers or by more careful selection than has been practiced in preparing this routine stock.

When these week to week deviations are smoothed out by averaging together the indices of several passages, there still remains a second component of the curve which shows a regular cyclic fluctuation with maxima lying just a year apart. The horizontal blocks in figure 1 indicate the positions of such means when groups of 26 passages beginning with passage 23 are so averaged. If a flattened sine curve be drawn with AA' as its axis, the wave

crests spaced 52 weeks apart, with minima ( $\sin \theta = -1$ ) falling on December 21, the curve will be seen to coincide rather closely with the observed growth rates, strongly suggesting that these fluctuations must follow some simple law related to the seasons.

Yearly cycles are, of course, common in the plant kingdom. In some cases these are obviously the result of the annual cyclic fluctuations of light and temperatures resulting from the alternation of seasons, and disappear under controlled environmental conditions. In others, *Asparagus medeoloides* Thunb., for example, the cycle is inherent and so firmly established in the make-up of the plant that in the northern hemisphere it continues to undergo its dormant period in May to September in spite of a completely inverted environmental cycle, and years of cultivation in the north will not alter this behavior pattern. It has seemed desirable to determine whether the cycle observed in these root-tips can be shown to be correlated with uncontrolled factors in the environment, or if it may be inherent.

The cultures in question were maintained under as uniform conditions as were available for large numbers. But, since they were kept in a laboratory room where other work was being carried on simultaneously, they were subject to two variables known to be only partially controlled and perhaps to other unknown ones. These two variables were light and temperature, both of which undergo seasonal cyclic fluctuations.

The cultures under consideration were at no time exposed to direct sunlight but, since they were placed in a well-lighted room, they received diffuse light of greater intensity and longer daily duration in summer than in winter. ROBBINS and MANEVAL (13), WHITE (15), and FELBER-PISK (3) had reported moderate illumination to be beneficial to similar cultures of root-tips of other plants, so that light might conceivably have been an important factor in determining the observed fluctuations. However, the studies of these authors were all of too short duration to be of undoubted significance. MALYSCHIEV, moreover, was unable to substantiate this conclusion (9). To test the question more thoroughly, a double-walled box was built of opaque matte-black cardboard, large enough to hold 25 cultures. It was so arranged as to allow free circulation of air, but light was completely excluded. Fifty cultures were prepared from passage 143, clone C. Twenty-five of these were placed in the dark box and set alongside of the other 25 which represented passage 144 of figure 1. Both sets of cultures were then transferred at weekly intervals for 10 weeks, being measured as usual at the beginning and end of each passage. Measurements and transfers of both "dark" and "light" cultures were made by daylight. The "dark" cultures were thus exposed to diffuse light for somewhat less than an hour each week, that is, about 0.5 per cent. of the elapsed time, while the "light" cultures were similarly exposed approximately 100 times this length of time (50 per cent.

of the elapsed time). The growth rates obtained are shown in figure 2. The mean difference in growth rates between the two sets for the entire 10 weeks was only 0.6 per cent., in favor of the "dark" cultures. This is too small a difference to be significant. It is worth noting, however, that had only passage 151 been measured, the result—a 40 per cent. increase of the "light" cultures over the "dark" ones—would have been in agreement with the findings of the earlier authors cited above, while observation of passage 152 alone would have led to an entirely contrary conclusion. The danger in

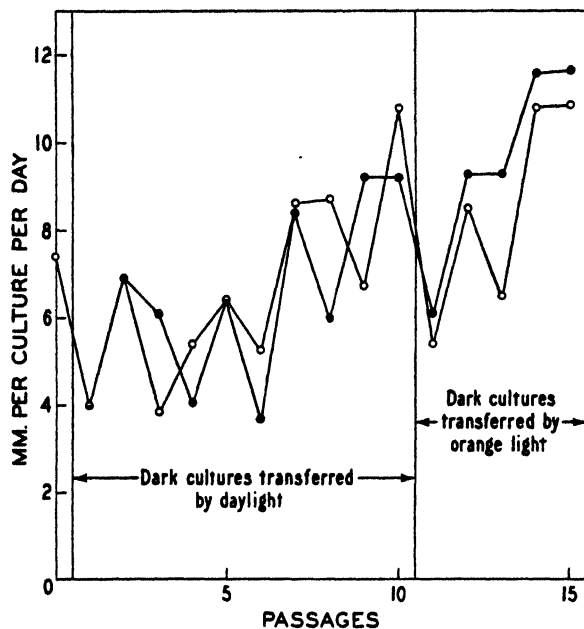


FIG. 2. Growth rates of cultures grown in diffuse daylight (open circles) and in darkness (solid circles) for 15 weeks.

drawing conclusions from single passages is thus evident. To make the result doubly sure, transfers were made for an additional 5 weeks, the "dark" cultures being transferred by the light of an orange Wratten Safe Light no. 0, without other exposure at any time. Throughout these 5 weeks (fig. 2), the cultures grown in the dark were consistently somewhat better than those provided with diffuse daylight. Since 15 weeks of greatly reduced illumination did not appreciably reduce the growth rates of these cultures nor cause any visible abnormalities in their behavior (fig. 3), it may safely be concluded that light is neither essential nor significantly beneficial to isolated tomato root-tips. Seasonal variations in illumination can, therefore, be ruled out as a factor in determining the cyclic variation in growth rate recorded in figure 1.



FIG. 3. Roots grown in diffuse daylight (two at left) and in continuous darkness except for the time required in transferring (two at right). The photograph was taken at the end of the 15th weekly passage under these conditions.  $\times 0.6$ .

The room in which the cultures were maintained was thermostatically controlled as to *minimum* temperature, so that the temperature never fell below  $20^{\circ}\text{C}$ . Since, however, no provision was made for cooling when the outside air temperature exceeded this figure, the room temperature sometimes rose to  $32^{\circ}\text{C}$ . WHITE had earlier shown (15) that isolated wheat roots are sensitive to variations in temperature, growing best at about  $27^{\circ}\text{C}$ ., so that this also might have been an important factor in determining the observed cycle.

Had light proved to be a significant factor in determining growth rates, the exact evaluation of temperature effects would have been difficult, since, in order to obtain comparable results, it would have been necessary to maintain a uniform light intensity at all temperatures studied. Since, however, variations in illumination over a rather wide range of intensity are without significant effect, this factor can be ignored in temperature studies so long as the light intensity is kept low. To test the effects of temperature variations, constant temperature ovens providing no illumination and controllable to  $\pm 2^{\circ}$  were used for temperatures  $5^{\circ}$ ,  $8^{\circ}$ ,  $10^{\circ}$ ,  $15^{\circ}$ ,  $20^{\circ}$ ,  $25^{\circ}$ , and  $28^{\circ}\text{C}$ . Temperatures of  $30^{\circ}$ ,  $31^{\circ}$ ,  $32^{\circ}$ ,  $33^{\circ}$ ,  $35^{\circ}$ , and  $40^{\circ}\text{C}$ . were provided in a water bath which admitted a low intensity of light to the cultures and was controllable to  $\pm 0.5^{\circ}$ . Twenty cultures were grown at each temperature, and measurements made at the end of one week. The mean growth rates for single passages at these temperatures are shown in figure 4. They are to be compared with those shown in figures 2 and 3 in the author's earlier work on wheat root-tips (15). The optimum at  $30^{\circ}$ —slightly higher than for

wheat—is extraordinarily sharp, an increase in maintained temperature from 28° to 30° causing a 20 per cent. increase in growth rate, while a further temperature increase from 30° to 31° caused a 30 per cent. drop in growth rate. Unlike variations in illumination, variations in temperature are obviously of great importance in determining growth rates.

The range of temperatures to which the cultures, whose growth rates are shown in figure 1, were subjected was, as has been said, from 20° to 32° C. As shown in figure 4, this is sufficient to determine a range of mean growth

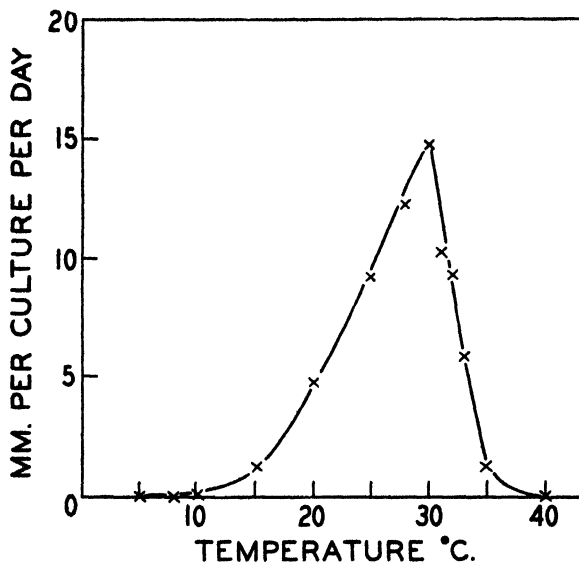


FIG. 4. Graph showing the relation between mean growth rate and maintained temperature, recorded over a single passage.

rates from 5 mm. per culture per day (at 20°) up to 15 mm. per culture per day (at 30°) and then back to 9 mm. per culture per day at 32°. Cultures grown at 32° were of poor color, often misshapen, and obviously in unsatisfactory condition. If the growth rates recorded in figure 4 be compared with the mean growth rates for the various seasons, as shown in figure 1, it will be seen that the growth rate for 20° C.—5 mm. per culture per day—corresponds very closely to the rates obtained during three consecutive winters—5.1, 5.1, 6.0—when, owing to low outdoor temperatures, it was possible to maintain the room temperature at 20–22°. The growth rate for 24°, which may be considered an average room temperature for summer,—8 mm. per culture per day—corresponds closely to the rates observed during the three summers covered by the record—7.2, 7.7, 7.2. Moreover, the maximum growth rate recorded in figure 4—15 mm. per culture per day at 30° C.—differs only slightly from that recorded in figure 1, 13.4 mm. per

culture per day in June, 1935, at a time when the mean temperature of the culture room may easily have approached such a value.

From the figures presented above it is evident that variations in temperature of the range to which the cultures under consideration are known to have been subjected, a range characteristic of a moderately well controlled laboratory, are quite sufficient to explain the cyclic fluctuations recorded in figure 1. Whether this is, as the data seem to indicate, the sole explanation or whether, when this variant is eliminated by more careful control of the temperature, there will remain a residual cyclic variation which is inherent, must await further study over an additional period of a year or more. The record presented here, however, serves to emphasize what has been repeatedly noted before, that growth which is sufficiently active for experimental purposes and which is reproducible can be obtained with root-tip materials only when all cultural conditions are rigidly controlled. Sensitivity to small variations in temperature and to slight variations in the concentrations of many of the nutrient ions must be taken into consideration in the interpretation of all results. The importance of these particular variables can hardly be over-emphasized; but as is evident from the results with various intensities of illumination, there also exist variables which are not important.

### Summary

The growth rates of a clone of isolated tomato root-tips cultivated *in vitro* and measured at weekly intervals for a period of 3 years show a random fluctuation around a mean due to variations in the behavior of the individual cultures. In addition, these growth rates show a cyclic fluctuation correlated with the seasons of the year. Investigation of the effects of light *vs.* darkness showed that seasonal fluctuations in illumination are a negligible factor in producing this cycle. Investigation proved, however, that such cultures are very sensitive to temperature differences and that the observed fluctuations in temperature are sufficient to account for the observed seasonal variations in growth rate. It will probably be necessary to control the room temperature more accurately than has been done in the past if uniform cultures are to be maintained throughout the year.

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# STRUCTURAL CHANGES PRODUCED IN LEAF TISSUE OF SOY BEAN PLANTS BY IRRADIATION OF THE DRY SEEDS WITH SOFT X-RAYS

THEO. P. LONG AND H. KERSTEN  
(WITH EIGHT FIGURES)

## Introduction

In a recent paper NOGUCHI (2) described histological studies made on the structure of Russian sunflower leaves grown from seeds irradiated at the stage of incipient germination, with hard, or short wave-length, x-rays. This paper describes a similar study in which the seeds used were dormant soy beans and the radiation was soft, or long wave-length, x-rays generated by a gas x-ray tube (1). Although it is not the purpose of this paper to compare the two types of radiation with regard to their ability to produce deformed plants from irradiated seeds it may be said that if a given quantity of radiant energy in the x-ray region here considered should strike the surface of a seed, a greater part of it would be absorbed by the seed if the radiation were of long wave-length than if it were of short wave-length. If the extent of the deformity produced in the plants grown from x-irradiated seeds depends on the quantity of energy they have absorbed, then one would expect the longer wave-length to produce the greater deformity, if the incident quantities of energy were alike.

## Method

Wilson black soy bean seeds which had been stored under ordinary conditions of temperature and humidity for about a year were used. They had therefore acquired the moisture content normally associated with dormant seeds. These were placed 8 cm. from the focal spot of the x-ray tube and were irradiated for periods from 10 to 80 minutes with the x-rays from the tube mentioned, when it was operated at 20 peak kv. and 10 m.a. Under these conditions the shortest wave-length in the beam of x-rays was about 0.6 Å., while the most intense part of the beam was the K<sub>α</sub> line of copper (1.54 Å.). The beans were then planted in ordinary soil in a greenhouse, together with unirradiated ones for controls. The irradiated seeds produced deformed plants, of which figure 1 is an example, while the unirradiated seeds produced normal plants. Those which were irradiated for the longer periods of time produced plants exhibiting similar deformities to a greater degree.

## Results

After an examination of the outward appearance as well as the corresponding sections of a large number of deformed leaves, it was found that almost all of them fell into one of the following classes:



FIG. 1. Soy bean plant with injured leaves.

1. Normal leaf (fig. 2). The epidermal cells are of uniform rectangular shape and form a fairly smooth leaf surface. The palisade layer is composed in most cases of two layers of cells placed evenly and having only a small amount of space around each cell. The chloroplasts are arranged just inside the cell wall and are spaced evenly. In the spongy parenchyma layer the cells show an irregular pattern in cross-section and have the chloroplasts arranged irregularly. The spongy tissue extends all the way to the epidermis.



FIG 2. Normal leaf

2 Leaf with numerous small light spots (fig 3). These spotted areas are caused by the failure of the upper layer of palisade cells to develop



FIG. 3. Leaf with numerous small white spots.

normally and produce chloroplasts, so that these areas are light in color. Sometimes the second layer of palisade cells is also affected. Neither the epidermis nor the spongy tissue is noticeably changed.

3. Leaf with large light-grey areas, found mainly on the second and third sets of leaves (fig. 4). In this case the palisade cells appear to be



FIG. 4. Leaf with light green area

normal but are spaced farther apart. In some leaves there are fewer than the normal number of chloroplasts in the cells. The chloroplasts in the cells of the spongy parenchyma layer are arranged irregularly, sometimes clumped together.

4. Leaf with small dark-green areas which appear normal except for color (fig. 5). (The dark-green areas are difficult to show in photographs of the leaves.) This injured condition seems to be due to an unusually large number of chloroplasts containing an excessive amount of chlorophyll in both the palisade cells and in the spongy tissue.

5. Leaf with large dark-green areas often raised and crinkled (fig. 6). In this kind of tissue the palisade cells are closely packed together and the cells seem to have more than the usual number of chloroplasts. The palisade



FIG. 5. Leaf with small dark-green areas.

cells are usually somewhat elongated and extend into the spaces belonging to the spongy parenchyma. The spongy tissues are smaller in volume, and the cells adjacent to the lower epidermis are often arranged as a layer of short palisade cells containing many chloroplasts. The epidermis, especially the upper layer, is usually irregular with more rounded and otherwise oddly shaped cells. The leaves from which these sections were made received illumination from above, while growing, just as those exhibiting the other types of deformity. In both types 4 and 5, some of the cells contain chloro-



FIG. 6. Leaf with large dark-green areas.

plasts which are of a dark blue-green color suggesting a possible abnormal percentage of chlorophyll 'a' and 'b.'

6. Leaves with white areas, either albino patches on the green leaf, or an entirely white leaf (fig. 7). In the albino leaves the palisade cells are



FIG. 7. Albino leaf.

entirely absent in the specimen examined. Only one plant exhibiting marked albino characteristics was grown (fig. 8). It grew from seed which had been irradiated 10 minutes at 35 peak kv. and 20 m a , placed 30 cm. from the focal spot. The albino characteristics may have been due to factors other than the x-irradiation but sections of its leaves are interesting when compared with the types of injury which can be produced at will. The spongy tissue seemed normal except for the absence of almost all the chloroplasts, while those present contained little or no chlorophyll. The epidermis was composed of rounded and oddly shaped cells. The veins were abnormally near each other and stood out as ridges. The intervacular part of the leaf was about  $60\ \mu$  thick compared with about  $160\ \mu$  for a normal leaf. The tissues were very difficult to stain and photograph.

7. Leaf with areas completely absent. (Several examples are shown in fig. 1.)

The authors wish to thank Dr. J. HOBART HOSKINS, and Dr. F. M. TURRELL of the Botany Department for their interest and assistance.



FIG. 8. Plant showing marked albino characteristics.

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# NUTRITIONAL STUDIES ON LOBLOLLY PINE

RUTH M. ADDOMS

(WITH TWO FIGURES)

## Introduction

The investigation herein reported was undertaken as part of a cooperative project on the biology of forest trees at Duke University. It represents an attempt to add a contribution to knowledge of the mineral nutrition of certain forest trees, in the hope that ultimately it may be possible to correlate the findings with studies of soil composition and with the local distribution of these tree species. Loblolly pine (*Pinus taeda* L.) was selected as a species that is relatively cosmopolitan, and yellow poplar (*Liriodendron tulipifera* L.) as a species that is relatively specific in environmental requirements. The present paper is a preliminary report on loblolly pine only.

For the purposes of this investigation, sand cultures were regarded as the most desirable method. Of the extensive work with sand cultures by many investigators within the past few years, little has been concerned with woody plants, and very little with forest trees. MITCHELL'S (4) experiments on Scotch pine and white pine in the Black Rock Forest represent the most fruitful work of the kind that has been done with forest trees.

## Experimental methods

Loblolly pine was grown in sand cultures for periods as long as 29 months. The first experiment was started in February, 1934, when 50 seedlings, 8 months old, were transplanted from a sand-peat mixture to washed white quartz sand in ordinary 10-inch unglazed pots. Of these seedlings 30 were transplanted in January, 1935, to sand in 3-gallon glazed crocks with drainage at the bottom. Nutrient solutions were added at first twice and later three times per week; at all other times the moisture content was maintained by the addition of distilled water. From time to time individual plants were removed from the series for study. Four of the 22 plants that remained on July 14, 1936, are shown in figure 1.

Other series were grown for shorter periods of time, employing similar methods except that glazed self-draining pots were used from the start.

Another series of loblolly pine was grown in sand culture for 14 months under slightly different conditions. The same type of crocks and the same solutions were used, but the solutions were applied daily by a drip-method of renewal. Addition of nutrients was not continuous, but 10 pots set up in series received each day 18 liters of solution, which dripped from capillary tubes. The arrangement of apparatus is shown in figure 2. In this series, one



FIG. 1. *Pinus taeda* L. grown for 29 months in sand culture. For two trees on left, nitrogen was supplied as calcium nitrate; for two trees on right, as ammonium sulphate.



FIG. 2. Drip-cultures at the end of 14 months. Capacity of each carboy is 18 liters. Uniform rate of drip is maintained by means of bottles that serve as constant-level reservoirs. Solution reaches individual cultures by dripping from capillary tubes. For series on left, nitrogen was supplied as calcium nitrate; for series on right, as ammonium sulphate.

set of 10 pots received its nitrogen from calcium nitrate, the other set from ammonium sulphate.

Since the experiments were primarily exploratory in nature in all series, the composition of solutions was varied from time to time in an attempt to determine the optimum conditions for growth. Concentrations of salts were varied as well as the hydrogen ion concentration, the latter by means of the addition of NaOH and H<sub>2</sub>SO<sub>4</sub>. The composition of two of the solutions that were successfully employed is given in tables I and II.

TABLE I  
COMPOSITION OF SOLUTION A

SALT	GRAMS PER LITER	CONCENTRATION IN P.P.M.	
		<i>p.p.m.</i>	<i>p.p.m.</i>
Ca(NO <sub>3</sub> ) <sub>2</sub> · 4H <sub>2</sub> O .....	<i>gm.</i> 1.148	Ca..... 195	N.....136
KH <sub>2</sub> PO <sub>4</sub> .....	0.643	K..... 184	P.....146
MgSO <sub>4</sub> .....	0.283	Mg..... 57	S..... 76
H <sub>3</sub> BO <sub>3</sub> .....	0.001	B..... 0.20	
MnSO <sub>4</sub> .....	0.001	Mn..... 0.40	
FeCl <sub>2</sub> .....	0.002	Fe..... 0.70	

TABLE II  
COMPOSITION OF SOLUTION B

SALT	GRAMS PER LITER	CONCENTRATION IN P.P.M.	
		<i>p.p.m.</i>	<i>p.p.m.</i>
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	<i>gm.</i> 0.734	N..... 156	S.....156
KH <sub>2</sub> PO <sub>4</sub> .....	0.870	K..... 249	P.....198
MgSO <sub>4</sub> .....	0.167	Mg..... 33	S..... 45
CaCl <sub>2</sub> .....	0.324	Ca..... 117	Cl.....204
H <sub>3</sub> BO <sub>3</sub> .....	0.001	B..... 0.20	
MnSO <sub>4</sub> .....	0.001	Mn..... 0.40	
FeCl <sub>3</sub> .....	0.002	Fe..... 0.70	

### Results and discussion

The several experiments, extending over periods of varied lengths up to 29 months, proved to the satisfaction of the writer that (1) loblolly pine can be grown successfully in sand culture, and that (2) it can utilize nitrogen in the form of either nitrates or ammonium compounds. These two points will be discussed in some detail.

The pine trees grown in sand culture compared favorably with trees grown in soil as to size and general appearance, with the exception of color

as discussed below. One difficulty that was experienced in sand culture, however, was the inequality of growth of the different individuals in a series. All of the plants grew, but the amount of growth varied greatly. When the number of individuals in a series must be limited, this factor is important. MITCHELL (4) refers the difference in his seedlings to difference in seed weight, and cites correction factors. In the present experiments an attempt was made to obviate the difficulty by using seedlings several months old and selecting them for uniformity of size and general appearance. The results indicate, however, that such selection is not sufficient. Figure 2 shows that the size of plants after several months of identical cultural treatment varied greatly. This difference was obviously the result of differences in plants and not of differences in treatment. In experiments with plants that can be propagated vegetatively, it would probably be better to work with rooted cuttings than with seedlings.

A second difficulty was the yellowing of leaves that was apparent from time to time in both nitrate and ammonium series. Usually it showed during the first few months after the seedlings were set out, and in most instances the plants subsequently recovered. The yellowing was much less noticeable in the drip cultures. In the light of MITCHELL'S (4) experience it seems reasonable to suppose that the cause was insufficient nitrogen. Mitchell states that the optimum concentration of nitrogen is 300 p.p.m., whereas in the solutions listed above, the concentration of nitrogen was only about half of that. In his experiments, however, the nutrient solution was added only twice during the 15 weeks of the experiments, whereas in the present experiments nutrient solution was added two or three times a week, and in the series of drip-cultures, every day. It is obvious, then, that the total amount of nitrogen supplied to the plants was considerably more than the concentrations indicate, in comparison with those of MITCHELL'S solutions. However, the fact that the pines of the drip-series showed much less yellowing than the others lends some evidence to the theory that the abnormality was caused by an insufficient supply of nitrogen. It was obviously not caused by deficiency of boron, manganese, or iron; iron was added in several forms and in several concentrations during the experiments, without apparent effect. Alteration of the hydrogen ion concentration from time to time during the course of the experiments had no appreciable effect on the yellowing. As stated above, most of the plants recovered and developed a healthy green color. Similar yellowing has been observed in soil cultures, and occasionally in the field.

Some of the seedlings developed a peculiar twisting and curling of the leaves that produced an appearance of knotting. This was observed in some but not all plants of every series, and in some plants more generally than in others. Eventually the leaves straightened and assumed a normal appearance. The abnormality was not restricted to these experiments, but was ob-

served in even more acute form in some soil cultures that were being conducted by another worker in the adjoining greenhouse. It has since been observed occasionally in pine trees growing in the field. The first explanation that suggested itself was that the sheath of the fascicle had become dry and hard, with the result that the normal elongation of leaves was mechanically restricted. In order to test this theory, several plants were placed for several weeks under bell jars, so that the humidity in which they were growing was greatly increased. Under this treatment leaves were as "knotted" as ever, disproving the theory.

These experiments showed conclusively that loblolly pine is capable of utilizing nitrogen in the form of either nitrates or ammonium. Alteration of the acidity over a wide range showed the best development with calcium nitrate as the source of nitrogen when the solution was decidedly acid (3.8 to 5 pH); with ammonium sulphate as the source of nitrogen when the solution was more nearly neutral (6 pH). The observations are entirely in keeping with those of TIEDJENS (6), TIEDJENS and BLAKE (7), TIEDJENS and ROBBINS (8), NIGHTINGALE (5), and DAVIDSON and SHIVE (2) on apple, peach, and other plants. It is altogether probable that under field conditions in the Piedmont of North Carolina loblolly pine receives most of its nitrogen in the form of ammonium compounds, for little nitrification occurs. In many forest stands of loblolly pine, as determined by COILE (1), the hydrogen ion concentration of the A horizon varies from 5.4 to 6.5 pH.

Although mycorrhiza were present when the seedlings were set out, they did not develop to any considerable degree in sand cultures. Field-grown plants show long white roots that are free from fungous infection and short, stubby, much-branched lateral roots that are infected with a tight mass of mycelium that penetrates the epidermis. Pines of the several experimental series also showed long white roots and short, branched lateral ones; however, the latter were not nearly so stubby as those of field-grown plants and microscopic observations showed no trace of fungous infection—any mycelium that was present was entirely external. Since rapid and apparently healthy growth was obtained in sand culture, it would seem likely that in field-grown loblolly pine the occurrence of mycorrhiza is incidental rather than highly beneficial.

The root systems developed in sand culture are worthy of mention, as their distribution is in rather striking contrast to that of loblolly pine roots under field conditions. In nature, most of the absorbing roots occur in the uppermost layers of the soil. In sand culture, however, the root systems practically fill the pots, and the absorbing roots are much more generally distributed. It is possible that the difference is attributable to the difference in aeration, and that the superficial character of the root systems of loblolly pines in many forest soils is caused by the deficiency of aeration in underlying horizons.

During the course of the present experiments, the trees were not dormant through a long period of time, such as the winter. In all of the series they showed during a year several periods of growth and several periods of dormancy, but never a long-continued winter dormancy. It is altogether possible that this condition is attributable to illumination. Although the pines were not intentionally given a longer photoperiod, other experiments in adjoining greenhouses were lighted regularly. It is possible that sufficient light reached the pines to prevent winter dormancy. WITHROW and BENEDICT (9) have shown that for certain plants an intensity of 0.3 foot candle is sufficient to produce a response. KRAMER (3) has demonstrated that it is possible to keep loblolly pines growing actively throughout the winter under greenhouse conditions.

In the light of these experiments the writer feels justified in pursuing the study of mineral deficiencies in loblolly pine by the use of sand cultures, employing either nitrates or ammonium salts as sources of nitrogen.

### Summary

1. Loblolly pine seedlings were grown successfully in sand cultures for periods up to 29 months.
2. They proved capable of utilizing nitrogen in the form of either nitrate or ammonium—the former more successfully in acid reaction, the latter more successfully in a more nearly neutral reaction.
3. The seedlings showed great individual variation in rate of growth even though they were selected on the basis of uniformity of size.
4. The root systems were deeper than those that are usually developed in the field.
5. Mycorrhiza were not formed.
6. Many plants of both the nitrate and the ammonium series showed, at times, a yellowing of leaves that may have been caused by an insufficient quantity of nitrogen. Apparently similar conditions were observed in soil cultures and to a lesser degree in the field.
7. Plants were grown throughout the year without long-continued periods of dormancy. This continuous activity may have been associated with a photoperiod longer than that of winter days.

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# EFFECT OF ETHYLENE CHLORHYDRIN AND THIOUREA ON *ELODEA* AND *NITELLA*<sup>1</sup>

BEATRICE MARCY

## Introduction

Ethylene chlorhydrin and thiourea were found by DENNY (4) to have the power of breaking the rest period of freshly-harvested potato tubers. DENNY (8), GUTHRIE, DENNY, and MILLER (6, 9), and MILLER (11, 12, 13) were unable to find any close correlation between the power of these chemicals to produce early sprouting and their effect upon respiration, glutathione or sulphhydryl content of the tissues, or enzyme activity, which might explain how dormancy is broken. The purpose of this study was to see if ethylene chlorhydrin and thiourea produced any change in the rate of the streaming of protoplasm, a change which might indicate their effect upon protoplasm itself, and so throw some light upon the manner in which dormancy is broken by them.

## Method

The plants used were *Nitella* sp., originally collected near Northampton, Mass., and *Elodea canadensis gigantea* (*E. densa*), obtained in January, 1936.

The chemicals, ethylene chlorhydrin and thiourea, were obtained from the Research Laboratory of the Eastman Kodak Company. Distilled water from a zinc still was used for all solutions. The concentrations of the solutions were 10 to 0.025 gm. per liter. Check tubes containing distilled water, but no chemical, were included with each set of experiments. Some additional measurements were made for each species in tap water.

For each test, 30 cc. of each solution were used. About four inches of the terminal portions of plants of *Elodea*, and of filaments of *Nitella* were placed separately in cotton-stoppered test-tubes containing the solution, and arranged so that all were equally lighted from an adjacent window. The temperature, at the time of taking the readings, varied at most 5° C. At 2-, 24-, and 48-hour intervals, one leaf was detached from each *Elodea* shoot. This leaf was always from among the lower leaves of the terminal bud. Rate of streaming in millimeters per second was measured with a standardized ocular micrometer, and a standardized metronome. From each leaf ten readings were made, from adjacent cells at the base of the leaf, near the midrib. For *Nitella*, readings were taken, as much as possible, from cells similar in size and age. Thus, there were tested two chemicals, each in ten dilutions, used

<sup>1</sup> This paper was presented before the New England Section of the American Society of Plant Physiologists at Storrs, Connecticut, May, 1936.

on two species, and treated for three different lengths of time. Each reading was checked by ten counts, and the entire experiment was done five times.

### Experimental results

Both ethylene chlorhydrin and thiourea, in concentrations of less than 1 gm. per liter, increase the rate of streaming of the protoplasm of *Elodea* and *Nitella* after these plants have been treated for 2, 24, or 48 hours. A solution containing 50 gm. per liter of either chemical causes the death of the cells of both plants after 24 hours' treatment (tables I-IV).

When *Elodea* is treated with ethylene chlorhydrin for either 24 or 48 hours, the point of maximum streaming falls at a concentration of 0.1 gm. per liter or less (table I). Concentrations higher than this cause a decrease in the rate of streaming, movement being completely stopped at 10 gm. per liter. However, with only 2 hours' treatment the maximum rate is at a concentration of 10 gm. per liter, and the lower concentrations have not affected the rate of streaming in any significant way (table I). It appears that in 2 hours enough chemical to effect the protoplasm is absorbed only from the 10 gm. per liter solution.

TABLE I  
*ELODEA* TREATED WITH ETHYLENE CHLORHYDRIN

CONCENTRATION IN GRAMS PER LITER	RATE OF STREAMING*		
	2 HOURS	24 HOURS	48 HOURS
gm.	mm./min.	mm./min.	mm./min.
10.000	0.5388†	0.0000	0.0000
0.750	0.2256	0.2880	0.2686
0.500	0.2484	0.3384	0.2460
0.250	0.2868	0.3805	0.3433
0.100	0.2797	0.4380†	0.3900
0.075	0.2856	0.3960	0.3949†
0.000	0.2616	0.2412	0.2208
Maximum percentage of increase	100.59	81.6	78.84

\* Each figure represents the average of 50 readings.

† Maximum rate recorded.

When *Elodea* is treated with thiourea for 24 or 48 hours, the effect is similar, except that streaming is not altogether stopped by a solution of 10 gm. per liter (table II). The maximum rate is induced by a concentration of 0.1 gm. per liter. The rate, after 2 hours of treatment, is greatest at a concentration of 0.5 gm. per liter, but the difference between the effects of the different concentrations is not marked. Again, the rates of streaming in various concentrations after 24 and 48 hours are very similar for the two chemicals.

TABLE II  
*ELODEA* TREATED WITH THIOUREA

CONCENTRATION IN GRAMS PER LITER	RATE OF STREAMING*		
	2 HOURS	24 HOURS	48 HOURS
<i>gm.</i>	<i>mm./min.</i>	<i>mm./min.</i>	<i>mm./min.</i>
10.000	0.3360	0.2473	0.1380
0.750	0.3660	0.3636	0.3468
0.500	0.4332†	0.4068	0.3756
0.250	0.3150	0.4596	0.4093
0.100	0.3890	0.4968†	0.4620†
0.075	0.3420	0.4356	0.3856
0.000	0.2385	0.2269	0.2424
Maximum percentage of increase	81.64	119.0	90.51

\* Each figure represents the average of 50 readings.

† Maximum rate recorded.

The effect of ethylene chlorhydrin and of thiourea upon the rate of streaming of the protoplasm of *Nitella* is similar to their effect upon *Elodea*. In a graded series of concentrations of ethylene chlorhydrin, the rate of streaming of *Nitella* cells rises to a maximum at 0.25 gm. per liter after 2, 24, and 48 hours of treatment (table III). After 24 hours in a 10 gm. per liter solution of ethylene chlorhydrin the protoplasm of *Nitella*, like that of *Elodea*, has ceased to stream. The highest rate of streaming was obtained after a 24-hour treatment, although 2 hours produced almost as high a maximum rate.

TABLE III  
*NITELLA* TREATED WITH ETHYLENE CHLORHYDRIN

CONCENTRATION IN GRAMS PER LITER	RATE OF STREAMING*		
	2 HOURS	24 HOURS	48 HOURS
<i>gm.</i>	<i>mm./min.</i>	<i>mm./min.</i>	<i>mm./min.</i>
10.000	1.494	0.000	0.000
0.750	2.310	1.963	1.925
0.500	1.971	1.946	2.130
0.250	2.669†	2.976†	2.241†
0.100	2.432	2.610	2.111
0.075	2.077	2.049	2.010
0.000	1.359	2.006	1.374
Maximum percentage of increase	99.24	48.37	63.1

\* Each figure represents the average of 50 readings.

† Maximum rate recorded.

The effect of thiourea upon the rate of streaming of *Nitella* may be shown by a curve very similar to that of *Elodea*, the maximum rate occurring at 0.1 gm. concentration in both species (tables II, IV). As the concentration of the solutions increased, the rate of streaming decreased, but not to zero. The 2-hour treatment, however, showed a maximum in the curve at 0.075 gm., then decreased (table IV). Both ethylene chlorhydrin and thiourea in-

TABLE IV  
*NITELLA* TREATED WITH THIOUREA

CONCENTRATION IN GRAMS PER LITER	RATE OF STREAMING*		
	2 HOURS	24 HOURS	48 HOURS
gm.	mm./min.	mm./min.	mm./min.
10.000 .....	0.923	1.224	1.744
0.750 .....	1.305	2.158	1.957
0.500 .....	1.385	3.052	2.246
0.250 .....	1.164	1.792	2.152
0.100 .....	1.639	2.600†	2.656†
0.075 .....	1.813†	2.391	2.215
0.000 .....	0.894	1.532	1.349
Maximum percentage of increase	102.8	69.72	96.87

\* Each figure represents the average of 50 readings.

† Maximum rate recorded.

crease the rate of streaming in *Nitella* in a shorter time than they do in *Elodea*.

The fact that the rather acid distilled water (pH 5.6), used in making up the solutions, does not affect the streaming of protoplasm is shown by a comparison of readings taken from plants in distilled water with those taken from plants in tap water (table V).

TABLE V  
*ELODEA* AND *NITELLA* IN WATER

RATE OF STREAMING				
PLANT	TAP WATER	DISTILLED WATER		
		2 HOURS	24 HOURS	48 HOURS
		mm./min.	mm./min.	mm./min.
<i>Elodea</i> .....	0.2100	0.2616	0.2412	0.2208
	0.2004	0.2385	0.2269	0.2424
<i>Nitella</i> .....	1.894	1.5590	2.006	1.3740
		0.8940	1.532	1.3490

### Discussion

The concentration of the solutions used in this experiment are necessarily far weaker than those used by DENNY (4) in his work on the potato, because of the more delicate nature of the material used in this experiment and the method of treating it. That the rate of streaming of the protoplasm of *Elodea* and of *Nitella* is increased by treatment with ethylene chlorhydrin and thiourea implies that these chemicals must change the protoplasm in some way. In the earlier work of SEIFRIZ (16, 17, 18), MAXIMOV (10), NADSON and MEISEL (14), NICHOLS (15), and WEBER (19) it was found that substances increasing the rate of streaming of protoplasm also decrease its viscosity, probably because they increase the dispersal of the colloidal particles in protoplasm. Further experimental work must be done before it is possible to say that the chemicals used in this experiment have this effect upon protoplasm, and in this way help to bring about the breaking of dormancy.

### Summary

1. Both ethylene chlorhydrin and thiourea in concentrations of less than 1 per cent. and 5 per cent. respectively, after 24 and 48 hours' treatment, increase the rate of streaming of the protoplasm of *Elodea* and *Nitella* cells.
2. Concentrations of more than 1 per cent. ethylene chlorhydrin and 5 per cent. thiourea are toxic to *Elodea* and *Nitella*.
3. The maximum rates of streaming are produced by a solution of 0.01 per cent. ethylene chlorhydrin in *Elodea*, and of 0.025 per cent. in *Nitella*; and by a solution of 0.01 per cent. thiourea in *Elodea* and *Nitella*. The maximum increase in the rate of streaming of treated over untreated protoplasm averages 70 to 100 per cent.

The writer wishes to express her gratitude to Dr. HELEN A. CHOATE for suggesting the problem, and to Dr. CHOATE and Dr. DOROTHY DAY for their kind assistance in carrying out the work.

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## BRIEF PAPERS

### SPECTROGRAPHIC METHOD FOR DETERMINING THE CARBON DIOXIDE EXCHANGE BETWEEN AN ORGANISM AND ITS SURROUNDINGS<sup>1</sup>

(WITH TWO FIGURES)

The spectrographic method for the determination of carbon dioxide as here described makes use of the high opacity of  $\text{CO}_2$  gas to radiation in one of its fundamental infra-red absorption bands. The spectral region used is a band from 4.2 to 4.3  $\mu$ . Two millimeters thickness of pure  $\text{CO}_2$  at atmospheric pressure transmits only 22 per cent. of this radiation. This region was selected because water vapor and practically all other gases and vapors commonly present in air are transparent to this radiation.

Radiation of this wavelength is isolated from a suitable source (NERNST filament) by an infra-red spectrograph, and allowed to fall upon a vacuum thermocouple after passing through a known optical path length of air contained in an absorption tube. The apparatus is calibrated by filling the absorption tube with air free from  $\text{CO}_2$ , then with samples of known concentrations of  $\text{CO}_2$ , and the galvanometer deflections observed. For the range of concentrations from 0 to 0.1 per cent.  $\text{CO}_2$  an optical path length slightly under 1 meter is used. With this arrangement the sensitivity of the apparatus is such that a 1-mm. deflection represents one ten-thousandth of 1 per cent.  $\text{CO}_2$ . For most work half this sensitivity is ample, and it is easier to hold constant. Shorter path lengths enable the measurement of greater concentrations.

The particular advantages of this spectrographic method are: (1) the sensitivity is as great as that of the best chemical method, (2) it gives instantaneous measurements, (3) the measurements are independent of the humidity of the air and also independent of the presence of practically all other gases or vapors commonly found in air, (4) for ordinary room temperatures and pressures the apparatus has very small temperature and pressure corrections, and (5) it can be recalibrated in a few seconds.

There are two factors in this method which limit the speed of response. One is the thermocouple-galvanometer system which by some sacrifice in

<sup>1</sup> A rapid and sensitive spectrographic method of determining the carbon dioxide concentration of the air surrounding small living organisms has been developed by Dr. E. D. McALISTER, of the Division of Radiation and Organisms, Smithsonian Institution. It has proved so satisfactory in studying the carbon dioxide assimilation of young wheat plants that the Committee on Physical Methods deems it worth while to call attention to this unique method as a highly useful tool in studying some of the more intricate problems of photosynthesis and respiration of both plants and animals.—EARL S. JOHNSTON, *Chairman*.



sensitivity can be reduced to respond in about 1 second. The other factor is inherent in the growth chamber. In a closed system this depends upon how fast the air can be circulated from the plant or organism into the optical absorption tube to insure complete mixing. In a system devised for work on young wheat plants and provided with temperature and humidity control this was accomplished in about 20 seconds, as can be seen from figure 1. A

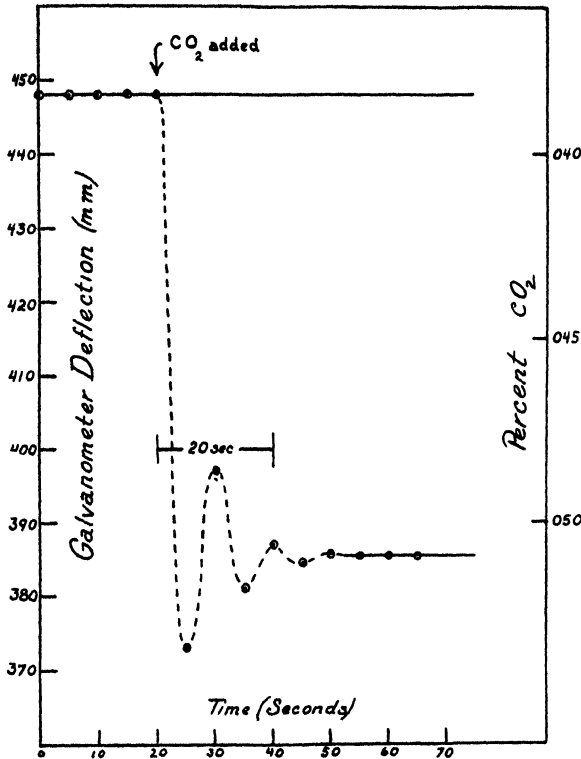


FIG. 1. Time response of the system.

small fan enclosed in the system causes the air to circulate once around the system in about 5 seconds. It can be seen from this figure that after about four trips around the system any change in  $\text{CO}_2$  concentration has been effectively equalized.

The success of this method depends upon the use of a sensitive vacuum thermocouple and a high-sensitivity galvanometer. (Leeds and Northrup H. S. galvanometer with scale at 5 meters was used in this work.) Its reliability depends upon a steady output of radiation from the source and upon the "zero" of the galvanometer-thermocouple system. In the present setup a shutter operated by a synchronous motor enables the operator to read the deflection and the zero point every 30 seconds. This eliminates zero

drifts but keeps the operator busy. The apparatus can, of course, be made self-recording. The freedom from any effect due to changes in humidity of the air in the system was demonstrated by blowing, first, air nearly saturated with water through the system, next, by blowing the same air through after most of the water was removed by passing it through sulphuric acid and noting that no measurable deflection resulted.

Figure 2 shows a typical set of data taken on 6 one-week-old Marquis

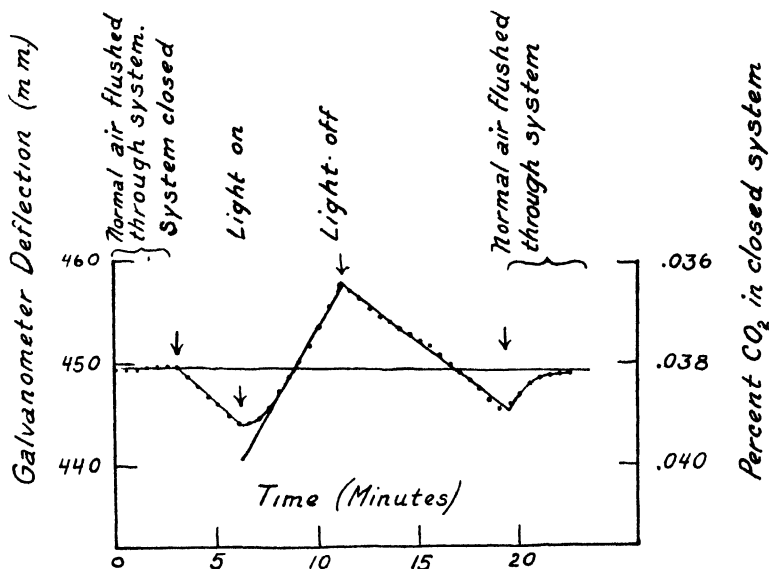


FIG. 2. Typical data on a green plant (Marquis wheat).

wheat plants. This shows how quickly the system follows the response of the plant. The short induction period seen in figure 2 during the first 2 minutes after illumination of the plants is surprisingly similar to that observed for algae by WARBURG, PAAUW, and others. It is to be noted, however, that by this method this induction period has for the first time been observed *directly* and not by the integration of the effect of a series of short exposures to light, each exposure being followed by a long period in darkness.—E. D. McALISTER, *Smithsonian Institution, Washington, D. C.*

CAROTENOIDS OF THE PEACH<sup>1</sup>

The two main problems involved in determining the nature of the carotenoid complex in a given material are, first, a thorough extraction of the pigments, and second, separation of the various components. In the case of the peach, the carotenoid concentration is definitely low, when compared with leaves, or with other fruits such as apricots, tomatoes, or peppers. The problem is further intensified by the difficulties inherent in drying this fruit without decomposition of pigments, and by the hygroscopic nature of the dried material.

Through the courtesy of Dry Fruit Products Incorporated, of Oakland, the writer was furnished with powdered vacuum-dried peaches (Muir variety), of which approximately 45 per cent. passed a 60-mesh sieve, and only this portion was used in the extraction. The material absorbed some moisture as a result of exposure during sieving, weighing, and transfer operations, and its moisture content was between 4 and 5 per cent. Of the 60-mesh material 2.5 kilograms were steeped in acetone overnight. A second steeping showed that the carotenoids had been almost completely (90–95 per cent.) extracted, although the powder was still definitely colored with pigments which could be extracted with aqueous alcohol, but which were not transferable to ether or petroleum ether.

For purposes of comparison, 4.5 kg. of fresh Lovell peaches which had been placed in cold storage (0° F.) were dehydrated and extracted by the usual wet extraction method with acetone.

In both cases, the carotenoids were transferred to petroleum ether (B. P. 30°–70° C.), which was then thoroughly washed with water, dried and concentrated under reduced pressure.

Passage of the petroleum ether concentrates over a TSWETT column of magnesia by the method of STRAIN (5) showed only minor differences, possibly of a varietal nature, in the relative amounts of the carotenoid components present. The Muir extract contained sufficient pigment for more detailed examination.

Development of the chromatogram gave four major zones, with a minimum of eight constituent carotenoids. The colored zone, least strongly adsorbed, consisted of  $\beta$ -carotene, with a very faint trace of the  $\alpha$ -component. This zone accounted for 35–40 per cent. of the total carotenoid coloring matter. Twelve mg. of recrystallized carotene were obtained from this zone. Absorption spectra maxima by the method of SMITH (3) in carbon bisulphide were 512 and 480 m $\mu$ , characteristic of  $\beta$ -carotene.

The second zone, from which 5 mg. of impure pigment were recovered,

<sup>1</sup> Contribution from the Division of Fruit Products, University of California, Berkeley.

accounted for approximately 25–30 per cent. of the coloring matter. It was identified as cryptoxanthin, absorption maxima in carbon bisulphide at 513 and 482 m $\mu$ . When mixed in equal proportions with cryptoxanthin from *Physalis alkekengi*, furnished by courtesy of Dr. STRAIN, it behaved as a homogeneous fraction when adsorbed on magnesia. Addition of  $\beta$ -carotene caused the formation of two zones on another test column.

From the third zone were obtained two pigments in amounts of less than 2 mg. When partitioned between petroleum ether and 90 per cent. ethanol, they remained quantitatively in the petroleum ether phase. After treatment, however, by refluxing with alcoholic KOH for thirty minutes, the main portion could be extracted with 90 per cent. ethanol. The absorption maxima of these two pigments in carbon bisulphide and in ethanol were those of lutein and zeaxanthin, indistinguishable from  $\alpha$ - and  $\beta$ -carotenes and their behavior toward solvents indicates that in the fruit they occur in an esterified condition.

The fourth and most strongly adsorbed zone, containing less than 10 per cent. of the color, on prolonged washing gave rise to three narrow bands. Absorption maxima were between those of lutein and zeaxanthin. Even after saponification, the pigments remained in the petroleum ether phase, when partitioned between this solvent and aqueous ethanol.<sup>2</sup>

### Discussion

So far as the writer can ascertain, no examination of the carotenoids of the peach has been made, with application of the TSWETT column technique. The investigations of STRAIN (6) have shown that the leaf xanthophyll complex consists of at least a dozen xanthophyll components, and it is becoming increasingly apparent that an equally complex carotenoid fraction exists in other parts of the plant, and that only the more abundant components have been at all adequately characterized.

It is of interest that whereas the pigments of the apricot (1, 2), according to the work of KUHN and BROCKMANN, consist largely of  $\beta$ -carotene,  $\gamma$ -carotene, and lycopene (all hydrocarbons in composition), those of the peach are (apart from the  $\beta$ -carotene fraction) largely xanthophyll in nature. The results reported by L. L. W. SMITH and O. SMITH (4) may be readily interpreted in accord with this. Studying a different problem, the effect of light on the carotenoids of certain fruits, they found a low concentration of carotenoids (1.9 mg. per kg. flesh) in the peach compared with 21.7 mg. in the apricot. When light was excluded from the fruit by bagging, there was a marked increase in the carotenoid content of the peach, and an almost equally striking decrease for the apricot. Red-fleshed varieties of tomatoes also had a lower carotenoid content when bagged. The apricot and the tomato both

<sup>2</sup> In this respect they differ materially from antheraxanthin and petaloxanthin (7).

contain lycopene, whereas the peach apparently does not. It seems not improbable that the increase in carotenoid content of the bagged peach noted by SMITH and SMITH is due to an increase in the esterified xanthophyll fraction, and the decrease in the other fruits due to their failure to develop lycopene in the absence of light. In any event, their results indicate fundamental differences in the pigments of the peach and apricot.

### Summary

Summarizing, we may conclude that the carotenoid fraction of the peach contains at least eight components. The four most abundant have been identified as  $\beta$ -carotene, cryptoxanthin, lutein, and zeaxanthin. Only a faint trace of  $\alpha$ -carotene could be found. The vitamin A potency of the peach will of course, therefore, reside in the carotene and cryptoxanthin fractions. No trace of lycopene or of  $\gamma$ -carotene was detected, in which respect the carotenoid complex of the peach differs materially from that of the apricot.—G. MACKINNEY, *University of California, Berkeley, California*.

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# BORON CONTENT OF APPLES AT DIFFERENT STAGES OF DEVELOPMENT

In view of the current interest in boron as one of the elements essential for the normal growth of plants, and as a factor concerned in the occurrence of physiological disorders of the apple, the writers have determined the boron content of this fruit at various stages in its development. The material used was fruit of the variety Golden Russet originally collected by one of us (DeLONG) for another purpose. This fruit was apparently normal in all respects and quite unaffected by any physiological disorders. Dates of collection and other descriptive data are given in table I. It should be noted that all of the samples are composite, approximately equal numbers of fruits having been gathered from each of several trees at each collection. These trees were of uniform age, all in a vigorous condition, and bore, in the season of collection, fair to good crops of fruit. The orchard soil is a sandy loam with a clay subsoil. The orchard is not cultivated, but the weed-grass growth is clipped in June. The chief fertilizer used is barnyard manure.

TABLE I

DATE OF COLLECTION	LABORATORY NUMBER	AVERAGE FRESH WEIGHT PER APPLE	DRY WEIGHT OF SAMPLE	NO. OF FRUIT IN SAMPLE	REMARKS
5/6/34 . . .	34-12	<i>gm.</i> 0.111	<i>gm.</i> 26.8	1000	Petals two-thirds fallen. Collected only fruit from which petals had dropped.
1/7/34 . . .	34-41	3.46	131.8	275	Starch beginning to appear in fruit.
31/7/34 .....	34-59	36.79	313.2	60	This and succeeding "A" samples consist of the parings of the sample. Prior to this date the fruit was ashed without paring.
5/9/34 .....	34-78	77.26	457.7	42	
	34-78A		119.4	42	
1/10/34 ....	34-109	102.2	603.0	40	
	34-109A		117.3	40	
15/10/34 .....	34-111	108.2	652.3	40	
	34-111A		113.1	40	

The analytical samples consisted of forty-gram portions of the dried tissue in each case, except that of no. 34-12, in which the total dry weight amounted only to 26.8 grams, and the whole amount was taken. These were

ashed in the presence of an excess of ten per cent. potassium carbonate solution at a temperature of about 450° C. with lixiviation. The method of analysis of the solutions so obtained was that described by SMITH.<sup>1</sup> The results obtained are given in table II.

These data indicate that in the healthy apple fruit the boron content progressively increases throughout the season. This increase is relatively very rapid for the period of active cell division and rapid growth, during the month of June, after which the rate of accumulation of this element becomes very much slower, although accumulation continues up to the time when the fruit is normally picked. In terms of parts per million of dry weight of tissue, however, the boron content shows a fairly rapid decrease during June and July, after which the amount present remains practically constant. When considered from either of the above viewpoints these re-

TABLE II

LABORATORY NUMBER	BORON PER APPLE	BORON, DRY WEIGHT BASIS
	<i>mg.</i>	<i>p.p.m.</i>
34-12 .....	0.00087	33
34-41 .....	0.0104	22
34-59 .....	0.079	15
34-78 .....	0.140	13
34-78A .....	0.043	15
34-109 .....	0.178	12
34-109A .....	0.044	15
34-111 .....	0.212	13
34-111A .....	0.043	15

sults emphasize the apparent importance of boron during the period when rapid growth of the fruit is taking place. The epidermal tissues, as represented by the parings, do not seem to vary appreciably in boron content from the flesh of the fruit, although they are possibly somewhat higher.—J. C. JOHNSON and W. A. DELONG, *Acadia University, Wolfville, Nova Scotia*.

<sup>1</sup> SMITH, G. S. The determination of small amounts of boron by means of quinalizarin. *Analyst* 60: 735-739. 1935.

## NOTES

**Thirteenth Annual Meeting.**—The thirteenth annual meeting of the American Society of Plant Physiologists was held at Atlantic City, December 29–31, 1936. The meeting was a very interesting and valuable one, and the program committee deserves great commendation for the manner in which a difficult situation was handled. The problem of accommodating the papers offered to the committee by a rapidly expanding membership becomes more severe every year. It is probable that the meetings will have to be extended to four days, or that the number of joint meetings held with other groups may have to be reduced if all papers are to be placed upon the program. The American Society for Horticultural Science has found a method of handling a very large number of papers in record time—138 papers in one day! They met in four sections with simultaneous meetings, and divided their papers into those to be presented by the authors, and those to be *read on demand*. One can imagine that the demand was not very great, with so many papers being presented in person! There may be much in this technique worthy of consideration by other groups harassed by too many papers for the short meeting periods.

The outstanding features of the program were the symposia on mineral nutrition including the minor elements, and on carbon dioxide assimilation. It is obvious, from comments overheard at the meeting, that programs of this type are of the greatest value to those who attend them.

The annual dinner was held on Tuesday evening, December 29, at the Hotel Dennis. The retiring president, Dr. A. E. MURNEEK, was unable to be present, owing to illness, and his address on recent advances in the physiology of reproduction in plants was read by Dr. OTIS F. CURTIS, vice-president of the Society. Other features of the dinner included the fifth award of the STEPHEN HALES prize, and the twelfth award of the CHARLES REID BARNES life membership, details of which are noted later. Two members of the society were elected as patrons in accordance with the constitutional provision for those who have contributed sufficiently to the financial support of the society. The secretary-treasurer made a very encouraging report which included the information that the society had passed the 500 mark in membership, and that library subscriptions had been notably increased during the year.

Atlantic City redeemed itself as a convention city in mid-winter. In 1932, the entire week was rainy; but the 1936 meetings occurred under almost ideal weather conditions. Only one day was rainy, and the week opened with weather warm and sunny enough for a summer day. Every-



thing considered, this meeting was one of the most satisfying ones in the history of the organization.

**Life Membership Award.**—The CHARLES REID BARNES life membership award was established at the Kansas City meeting in 1925, the second annual meeting of the American Society of Plant Physiologists, and the first award was made at Philadelphia in 1926. At the Atlantic City meeting in 1936, the twelfth award of this honor was made to Dr. DANIEL TREMBLY MACDOUGAL, pioneer among American plant physiologists, whose long career as Director of the Laboratory of Plant Physiology of the Carnegie Institution of Washington has been an inspiration to everyone. He published an early text book on plant physiology, second in point of time in America only to GOODALE's text, and in this way exerted a profound influence upon the development of experimental plant physiology in this country. He was a contemporary of Dr. BARNES, about six years younger than BARNES, and they were close friends. Dr. MACDOUGAL's contributions to the literature of plant physiology have been numerous, and are so well and favorably known that they need no enumeration. The society is to be congratulated upon the selection, and the committee of award, under the leadership of Dr. JOHN W. SHIVE, deserves great commendation for having exercised for the first time its highest constitutional privileges.

**Stephen Hales Award.**—The fifth award of the STEPHEN HALES prize was made to Dr. KENNETH V. THIMANN, formerly of the California Institute of Technology, now at Harvard University, for his contributions to our knowledge of the chemistry and physiological significance of the growth hormones of plants. He has also been a contributor of notable papers in the field of protein chemistry. Dr. THIMANN was born in England in 1904, and began to contribute actively to our knowledge of proteins at the age of 22. He received his training and degrees at the Imperial College, University of London (B.Sc., 1924; Ph.D., 1928), and distinguished himself as Frank Hatton prizeman in Chemistry in 1924. Later he was a demonstrator at the University of London, and held a Beit Memorial Research Fellowship in biochemistry from 1927 to 1929. In 1930 he was invited to join the staff of the William G. Kerckhoff Laboratories of the California Institute of Technology at Pasadena. Here he began his brilliant contributions to the field of plant hormones.

In the absence of the chairman of the STEPHEN HALES award committee, Dr. W. W. GARNER of the United States Department of Agriculture, the candidate was presented for the award by Dr. CHARLES A. SHULL. The certificate of award and the prize were conferred upon Dr. THIMANN by President R. B. HARVEY. A delightfully informal story of the career of

Dr. THIMANN was presented to the Society by Dr. GEORGE S. AVERY of the Connecticut College for Women.

**Rodney Howard True.**—In celebration of the seventieth anniversary of his birth, this number of *Plant Physiology* is dedicated to Dr. RODNEY HOWARD TRUE, who has been for many years professor of botany, director of the Botanic Garden, and Director of the Morris Arboretum at the University of Pennsylvania. Dr. TRUE was born at Baraboo, Wisconsin, October 14, 1866, won his earlier degrees at the University of Wisconsin, and his Ph.D. at Leipzig in 1895. He began his work in pharmacognosy at the University of Wisconsin, was lecturer at Harvard for a short time, and then plant physiologist in charge of physiological investigations in the Bureau of Plant Industry from 1902 to 1920. He went to the University of Pennsylvania from the U. S. Department of Agriculture in 1920. His contributions to our understanding of the relationships between root systems and the solutions in which they grow have been fundamentally very valuable. In other lands the "Festschrift" has been developed as a means of celebrating the seventieth birthday anniversary of those whose lives have been devoted to scientific research. The American Society of Plant Physiologists is happy to dedicate this number of *PLANT PHYSIOLOGY* to Dr. TRUE in celebration of his seventieth birthday anniversary, reached on October 14, 1936. With the dedication go the good wishes of all that Dr. TRUE may have many more years of happy and satisfying activity.

**Finance Committee Report.**—During the year 1936, two of the bonds possessed by the American Society of Plant Physiologists were called for redemption. One of these was a Texas Corporation bond, the other a gold debenture of the American Telephone and Telegraph Co. The funds rendered liquid by these calls have been reinvested, partly in a Texas and Pacific R. R. general and refunding bond, and partly in shares of Massachusetts Investor's Trust. The purchase of the latter investment was dictated by the present unhealthy condition of the bond market, and by the need of a hedge against a moderate inflation which has by no means yet run its course.

The Society's permanent funds amount to nearly \$8,000, with \$2,000 in the STEPHEN HALES endowment, \$3,000 in the CHARLES REID BARNES fund, \$1,400 in life memberships, slightly less than \$500 in the general endowment, and securities of unknown value in the reserve, represented by \$1,100 in real estate gold bonds. The first several funds are adequate to support their objectives at present, but the general endowment should be increased fifty-fold in order to contribute substantially to the publication program.

**Abstracts.**—Occasionally authors have failed or refused to submit abstracts of their papers appearing in *PLANT PHYSIOLOGY*. These abstracts are collected by the editor for use in *Biological Abstracts*. As long as this cooperation is needed by *Biological Abstracts*, it is the duty of authors to respond with an abstract typewritten on the blanks sent out with the galley proofs. The task of editing the papers used in *PLANT PHYSIOLOGY* has become so large that there is no time that can be devoted to writing repeatedly to delinquent authors. To clear this situation, the editor proposes to place with the printers of the journal more papers than are currently needed for each issue. When the forms must be closed, the issue will be made up of those papers whose authors have complied with the abstract requirement. The editor does not wish to assume the duty of even reminding authors that they have not sent an abstract. The abstract blank is supposed to be enclosed with the proofs, and is sufficient notice that it should be returned along with the corrected galleys.

**Methods in Plant Physiology.**—A laboratory manual and research handbook of methods in plant physiology was published on January 1, 1937, by the McGraw-Hill Book Co., New York. The authors are Dr. W. E. JONES, Iowa State College, and Dr. C. A. SHULL, University of Chicago. One chapter on statistical methods was contributed by Dr. GEORGE W. SNEDECOR, Iowa State College.

The first part of the book contains thirteen chapters, twelve of which are devoted to large sections of plant physiology, arranged in logical order. There are 182 experiments in all, and each experiment bears a designation to indicate whether it is elementary in character, intermediate, or advanced. The work can be adapted, therefore, to beginners, as well as to intermediate students, and to advanced or graduate students. The work can be made to serve as a laboratory manual at any level of training desired.

The second part is devoted particularly to methods useful in preparing for research in plant physiology. There is a chapter on general procedures, followed by six chapters dealing with analytical work. The first of these considers the collection of samples, killing, preserving, and extracting plant materials. Then follow chapters on the soluble carbohydrates, colloidal carbohydrates, soluble nitrogen, colloidal nitrogen, and plant ash. There is a chapter on physical measurements, and one on the measurement and control of environment. The final chapter is on statistical methods. An appendix provides 33 tables useful to the physiologist in his calculations. There are 472 pages, with the index, and the publisher's price is \$4.50.



THIS NUMBER OF PLANT PHYSIOLOGY  
IS DEDICATED TO  
FREDERICK FROST BLACKMAN  
IN CELEBRATION OF  
THE SEVENTIETH ANNIVERSARY OF HIS BIRTH  
JULY 25, 1866



*F. F. Blackman*

FREDERICK FROST BLACKMAN  
JULY 25, 1866



# PLANT PHYSIOLOGY

APRIL, 1937

## EFFECT OF LIGHT INTENSITY ON THE PHOTOSYNTHETIC EFFICIENCY OF TOMATO PLANTS<sup>1</sup>

ALTON MILLETT PORTER

(WITH ONE FIGURE)

### Introduction

The tomato stands foremost among the several vegetable plants which are cultivated as greenhouse crops. In its culture under glass, especially in the northern states, the question of sufficient light for its best development and highest productivity arises and becomes acute. The light of the natural day, during the winter months, appears to be inadequate with respect to its duration and also to its ordinary intensity.

Certainly photosynthesis is one of the most fundamental processes which condition plant behavior and production, and light is a major factor in its dynamic complex. Neglecting the characteristics of light, other than its intensity, how is its intensity related to the rate, the so-called efficiency, of photosynthesis? More particularly, what is this relationship respecting the tomato plant, when grown under greenhouse conditions? A study of this—induced by the desire to extend the knowledge disclosed by investigations already made and reported—was completed and is herein presented.

### Review of literature

The effects of strong, diffused light on photosynthesis were extensively studied by MUNTZ (10) in 1913. He found from field observations that alfalfa produced less dry matter per square centimeter of leaf area in the summer of 1911—a summer unusually free from clouds—than in 1910, when cloudy skies prevailed much of the time. Additional observations were made in the laboratory where it was possible to equalize the amounts of water received by the lots of plants grown under different light intensities. The

<sup>1</sup> Journal article no. 109 n.s., Michigan Agricultural Experiment Station, East Lansing, Michigan.



results of the laboratory experiments accorded with those obtained from the work in the field. He concluded that carbon assimilation is governed and limited by the intensity of the light.

LUBIMENKO (8) and POPP (15) found that in heliophilous plants the rate of the accumulation of elaborated materials was increased with increase in the light intensity, up to an optimum point, and that any increase beyond this optimum resulted in a decrease in the rate. Heliophobous plants behaved in the same manner, the optimum, however, being at a much lower point than that for the heliophilous types.

ARTHUR, GUTHRIE, and NEWELL (1), working with 30 different species of plants, found the tomato to be the most sensitive to light. Light intensities of 350, 450, 760, 800, 1200 and 1400 foot-candles were used conjointly with lengths of day which ranged from 5 to 24 hours. This revealed the fact that the "time factor" was of importance. The peak of increase in carbohydrate production was reached at higher light intensities with the 12-hour day. Injurious effects resulted when the day was lengthened to 17 and 19 hours. The maximum carbohydrate increase was reached with the 17- and 19-hour day when lower light intensities were used, or at the point of injury for the higher intensities.

COMBES (2), working with potatoes and other tuber-forming species, found that the higher the light intensity, the greater was the accumulation of elaborated organic compounds in the storage parts of the plants. Apparently, at lower intensities the storage function ceased and the entire amount of the products of photosynthesis was consumed in the growth of the aerial parts of the plant.

DEBESTEIRO and DURAND (3) obtained very definite results, experimenting with the garden pea. The plant's dry-weight increase was in direct proportion to the intensity of the light employed for its irradiation.

SMITH (18) and YOSHII (24) experimented with several of the different environmental factors, and, of these several factors, light intensity had the greatest effect on the production of carbohydrates in cereals and peas. Their data show a greater production of carbohydrates under the condition of short days with bright sunlight than that of long days with reduced sunlight, although the product of the intensity and the duration of light was higher in the latter case.

KOSTYTSCHEW and KARDO-SYSSOIEWA (7) found that desert plants increased in carbon assimilation up to an optimum light intensity and decreased as the intensity went above this point. Later in the day, as the light intensity fell to the optimum point, the carbon assimilation again reached a maximum causing the daily curve of photosynthesis to show two peaks in its outline.

The literature which bears directly on the question of the response of the photosynthetic function to the factor of light intensity is not plentiful. The

foregoing references are not all, but are representative of those of greater importance, and, also, are sufficient to show the existence of a quantitative relationship between these two phenomena.

Besides a direct effect of light, with respect to its intensity, upon the behavior of the photosynthetic process, acting as a catalytic and energizing agent, it appears to affect certain other factors, which are essential in the process. Among these are the chlorophyll content of the leaf and its anatomical structure; the latter being important with reference to the rate of the diffusion of gases into and within the leaf's interior.

WILLSTÄTTER and STOLL (23) observed that the rate of photosynthesis increased with the chlorophyll content, but were unable to establish a definite quantitative relationship between the two—the function and the independent variable.

PALLADIN (13) and LUBIMENKO (8) state, on the basis of their experiments, that heliophobous plants are relatively higher in chlorophyll content than heliophilous plants. The latter investigator was able to establish the fact that the optimum light intensity for photosynthesis is lower in correspondence with reduced content of chlorophyll. Shade plants at the lower light intensities were as efficient in photosynthetic activity as non-shade plants at these same light intensities.

A number of more recent investigators, JOHNSTON (6), MACDOUGAL (9), SPOEHR (19), and WIESNER (21), working with long day plants, report that the amount of chlorophyll in the leaves of plants increased in direct proportion to the average quantity of light received by them.

SPRAGUE and SHIVE (20) demonstrated that there was a degree of relationship between the total chlorophyll content and the dry weights of tops in corn. The total quantity of chlorophyll contained in the leaves of the various strains of maize correlated closely with their dry weights at successive harvests. Strains that showed a high chlorophyll concentration per unit of leaf area also had high average rates of increase in dry weights of tops, and *vice versa*. This ratio between the total chlorophyll and dry weight of tops was practically identical with all three strains of corn tested.

EMERSON (4), working with *Chlorella*, observed that plant cells low in chlorophyll reached their maximum rate of photosynthesis at approximately the same light intensity as normal cells. In working with different chlorophyll concentrations in plants that were kept constant in these variations, with the same light intensity, he found that the rate of photosynthesis increased at the same speed regardless of the chlorophyll concentration. The conclusion was that chlorophyll is probably a chemical reactant in photosynthesis as well as being the photosensitizer which absorbs the radiant energy necessary in the process.

HAYDEN (5) and POOLE (14) found the spongy parenchyma cells (mesophyll) of the leaves were poorly developed in sun plants, but in shade plants these cells replaced the palisade cells.

SHIBATA (17) observed that light intensity had a definite effect on the anatomy of the leaves, in that the epidermal cells are smaller in short day plants. OSTERHOUT (12), and NIGHTINGALE and MITCHELL (11) observed that leaves were thicker and had more elongated and more densely packed palisade cells as the average light intensity was maintained at a higher point.

The literature leaves no doubt concerning the direct, and also indirect, importance of light intensity in the plant's photosynthetic behavior. The results cited from the work of ARTHUR, GUTHRIE, and NEWELL (1) are especially significant and helpful, since the tomato plant itself was among those used in their experiments. However, additional contributions from controlled experimentation are desirable and necessary before the matter of the use of artificial light in forcing houses, devoted to tomato growing and production, can be certainly and soundly determined.

### Procedure

The tomato plants used in the experiment were of the Grand Rapids Forcing variety. The seeds were sown in greenhouse flats on January 26, 1933. On February 3 a large number of seedlings were selected and pricked off into two-inch pots. These were transferred on February 12 into four-inch pots and left therein until March 1, or until their development was such that they were ready for final transplantation. On that date, 36 of the plants were selected from the remaining 108, and transferred to 14-inch pots, in which they were grown singly and to full maturity.

The soil was a fairly rich orchard loam, which had been previously screened and thoroughly mixed, by having been shoveled over, in bulk. Its uniformity was as good as could be expected and secured.

The 36 plants were divided into three lots of 12 each, and each of the lots was placed, with wide spacing, on a separate greenhouse bench where the pots were surrounded by moist sand (afterwards kept moistened), to a depth of 5 inches. During the course of the experiment, the position of individual pots within each lot was systematically shifted twice each week. The number of clusters of fruit per plant was restricted to five.

A 14-hour day was maintained over the plants of each lot. Extension of the regular daylight period was accomplished by means of a 1000-watt electric lamp, with dome reflector and adjustable in height, suspended *centrally* above each group of plants. A wooden frame was constructed above each of two of the benches, under each light, and made to be vertically movable. One of these frames was covered with one layer of white cheesecloth, the other with two layers. This effected three respective intensities of the

light, both natural and artificial, for the plants: *no shade*, or *full intensity*; *one-half intensity*, 50.4 per cent.; and a little less than *one-fourth intensity*, 22.3 per cent. The shades were kept adjusted in their heights so as always to be approximately 24 inches above the tops of the growing plants.

It was aimed, of course, to keep the conditions of the environment, aside from the controlled variations in light intensity, the same for the three benches. Data were recorded for relative humidity, air temperature, and soil temperature, under each of the three light conditions, from March to July, by use of hygrothermographs and soil thermographs.

Additional information regarding methods—those more particularly technical—is given, where appropriate, in the following section, with its presentation of the data obtained.

## Experimental results

### GROWTH RESPONSE AND LEAF AREA

Possible relationships between each of several different linear measurements of the tomato leaf and its total area were examined in a previous experiment (16). The length of the leaf from the base of its first leaflets to the tip of the midrib proved to be the most accurate index. The type of association was clearly curvilinear, and, specifically, parabolic in the second degree. The derived equation was  $y \text{ (area)} = 3.16 + 0.417x + 0.307x^2$ .<sup>2</sup>

All of the leaves on each plant in each of the three lots were measured, in the manner indicated above, at intervals of three to seven days, and their areas calculated through the given equation. The data are presented in table I.

As shown by these data, expansion in leaf area was both continuously and finally the greatest for the plants under the lowest light intensity, next greatest where medium intensity prevailed, and least in the unshaded condition. The orderliness of the change in the daily rate of the increase in the foliar surface of the unshaded plants is outstanding. This rose over gradual steps to a distinct maximum (April 22), and thereafter fell off consistently to zero at the end. Differing from this, the other two maxima were reached more quickly and much earlier in the life of the plants, and were maintained over longer periods of time.

Thus, the usual result was obtained. Growth, when measured in terms of leaf area, augments under reduced light intensity. The leaves attain greater size, but commonly are thinner and may have even less total mass. The greater spread of leaf surface gives increased exposure to the light, such as it is, and tends in some degree to compensate on the whole for the lesser quantity of light received per unit of exposed surface.

<sup>2</sup> This leaf area equation was tested out on about 20 leaves from each of the three sets of plants and was found to apply equally well.

**TABLE I**  
GROWTH RESPONSE IN TERMS OF LEAF AREA

DATE OF MEASUREMENT	AVERAGE LEAF AREA PER PLANT			AVERAGE DAILY INCREASE IN LEAF AREA PER PLANT		
	NO SHADE	1 LAYER CHEESE-CLOTH	2 LAYERS CHEESE-CLOTH	NO SHADE	1 LAYER CHEESE-CLOTH	2 LAYERS CHEESE-CLOTH
	<i>sq. cm.</i>	<i>sq. cm.</i>	<i>sq. cm.</i>	<i>sq. cm.</i>	<i>sq. cm.</i>	<i>sq. cm.</i>
March 28 .....	1350	1503	1625	.....	.....	.....
April 4 .....	1649	2253	2473	42.7	107.0	116.0
April 8 .....	1888	2687	3122	59.7	105.8	162.1
April 11 .....	2088	2894	3594	66.6	100.4	157.1
April 15 .....	2358	3408	4202	67.5	107.5	152.0
April 18 .....	2668	3646	4694	102.8	77.7	158.5
April 22 .....	3188	3901	5294	130.0	63.6	147.5
April 29 .....	3754	4303	6259	80.7	57.3	139.0
May 6 .....	4164	4680	6649	58.5	56.1	55.6
May 13 .....	4325	5033	6764	23.0	48.0	16.4
May 20 .....	4485	5433	6868	13.3	57.0	14.8
June 5 .....	4563	5559	7072	6.5	7.9	12.7
July 3 .....	4563	5559	7072	.....	.....	.....

#### GROWTH RESPONSE AND STEM ELONGATION

The measurements taken for leaf area were accompanied by determinations which gave the growth rate of the main axis of the plants, under each light treatment. The distance measured was that of the stem axis. The period of these intermittent measurements was April 4 to June 5, when the plants were pinched out at the top, and thus restricted to the production of but five fruit clusters per plant. Table II gives the data.

The responses in stem elongation were akin to those shown for increases in leaf area. Growth in height, as reflected in more attenuated internodes, was more rapid as shading was heavier, and the plants taller at the time of being topped. The maxima for the rates of elongation were in the same order as those followed by the leaves, and their occurrences in time practically identical with those which obtained for the leaves.

#### GROWTH RESPONSE AND FRUIT PRODUCTION

These measurements were made by tagging each fruit of each cluster, giving the date of set, and recording the number of days necessary for it to attain its full weight. This information was assembled and totaled, giving comparative figures to illustrate the effect of light intensity on fruit set and production. The data are presented in table III.

The amount of fruit set under reduced light intensity is much less than under normal light. In the early part of the season when the plants were

TABLE II  
GROWTH RESPONSE IN TERMS OF STEM ELONGATION

DATE MEASURED	AVERAGE HEIGHT OF PLANT			DAILY INCREASE IN HEIGHT PER PLANT				INTERNODAL LENGTH		
	No SHADE	1 LAYER CHEESE-CLOTH	2 LAYERS CHEESE-CLOTH	No SHADE	1 LAYER CHEESE-CLOTH	2 LAYERS CHEESE-CLOTH		No SHADE	1 LAYER CHEESE-CLOTH	2 LAYERS CHEESE-CLOTH
April 4	cm. 45	cm. 61	cm. 70	cm.	cm.	cm.		cm.	cm.	cm.
April 8	50	68	76	1.25	1.75	1.50		4.8	5.5	6.2
April 11	54	73	87	1.33	1.66	3.66		4.8	5.5	6.0
April 15	59	82	95	1.25	2.25	2.00		4.8	5.6	6.2
April 18	64	94	100	1.66	4.00	1.66		4.5	5.9	5.7
April 22	70	96	105	2.00	0.50	1.25		4.7	6.7	5.9
April 29	74	98	112	0.57	0.28	1.00		5.1	6.5	6.2
May 6	78	100	116	0.57	0.28	0.28		5.2	6.4	6.5
May 13	81	102	117	0.42	0.28	0.14		5.2	5.9	6.6
May 20	81	102	117	0.00	0.00	0.00		5.4	6.0	6.2
June 5	81	102	117	0.00	0.00	0.00		5.4	6.0	6.2

**TABLE III**  
GROWTH RESPONSE IN TERMS OF FRUIT PRODUCTION

CLUSTER NO.	TOTAL NUMBER OF FRUITS			TOTAL WEIGHT OF FRUIT			AVERAGE WEIGHT OF SINGLE FRUITS			AVERAGE DAYS TO RIPEN, FROM SET		
	No SHADE	1 LAYER CHEESE-CLOTH	2 LAYERS CHEESE-CLOTH	No SHADE	1 LAYER CHEESE CLOTH	2 LAYERS CHEESE-CLOTH	No SHADE	1 LAYER CHEESE CLOTH	2 LAYERS CHEESE-CLOTH	No SHADE	1 LAYER CHEESE-CLOTH	2 LAYERS CHEESE-CLOTH
1	57	29	0	3335	1572	0	58.5	54.2	0	48.9	53.6	0
2	75	54	20	5866	3629	1555	78.2	67.2	77.7	47.7	49.6	54.7
3	58	57	39	5277	4062	2761	90.9	71.2	70.9	47.7	49.1	53.8
4	61	47	56	5797	3896	4590	95.0	82.9	82.0	46.0	48.0	53.6
5	20	18	27	1759	1448	1913	87.9	80.4	70.8	43.1	47.2	52.0
Totals	271	205	142	22034	14607	10819						
Av. per plant	22.5	17.0	11.8	1836.1	1217.2	901.5						
Av. per sq. meter of leaf area				7288	3894	2229						

receiving a relatively small amount of light the fruit set was in inverse proportion to all amounts of shading, but as the season progressed and light intensity became higher the fruit set correlated closely with the foot-candle hours of light (table VII) to which the plants were exposed.

Fruit production requires a greater area of leaves in proportion to the amount of shading the plants receive. The fruits attain greater weights and ripen sooner when the light intensity is not reduced.

#### GROWTH RESPONSE AND TOTAL PLANT PRODUCTION

The data for total plant production determined on twelve individuals under each of the three different light intensities are shown in tables IV, V, and VI. A cursory glance at these data shows that individual tomato plants vary within wide limits. The weights taken for total plant production show a variation in plant food under each light treatment that is less than the differences in light intensity.

In accordance with expectations, increases in light available for carbohydrate formation showed a greater quantity of fresh, dry, ash, and plant food weights in the average case. The rate of photosynthesis was slowed up according to the amount of light reduction in each block. The differences in light intensity appeared to have less effect on the ash content of the plants, but are in a rather definite relation with the average results on the fresh, dry, and plant food weights in all parts of the plants. The plant food manufactured per unit of leaf area is greatest under the no-shade condition and is reduced according to the amount of shade the plants receive. This efficiency in food manufacture seems to have a definite effect on the plant material used in fruit production and is in approximately the same ratio as the average decreased plant efficiency where shaded. It would seem from this information that differences in light intensity during the seasons of the year are the direct causes for variations in plant efficiency in growth and fruit production, but plant 10 in table IV, plant 11 in table V, and plant 11 in table VI are practically equal in their efficiency under each respective condition. This appears to indicate that plant variation is responsible for some of the differences in photosynthetic activity under the different light intensities.

#### ENVIRONMENTAL CONDITIONS

The experimental aim, as stated earlier, was to have the same length of day (14 hours) for the three lots of plants, while having them exposed to three different light intensities. Light measurements were made daily, at two-hour intervals, throughout the period of growth, by means of a Clements' photometer. The data for these measurements are presented in table VII.

It is clear, from examination of table VII, that the graduations of light intensity, established in the beginning by means of shading, were maintained



TABLE IV  
GROWTH RESPONSE IN TERMS OF TOTAL PLANT PRODUCTION BY PLANTS NOT SHADED

PLANT	FRESH WEIGHT				DRY WEIGHT				ASH WEIGHT				PLANT FOOD WEIGHT*			
	Tops	Roots	Fruit	Total	Tops	Roots	Fruit	Total	Tops	Roots	Fruit	Total	Tops	Roots	Fruit	Total
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
1	629	131	2217	2977	91	22	11	226	16.4	7.9	7.9	32.2	74.6	14.1	105.1	193.8
2	382	42	1823	2247	58	8	93	159	12.2	1.6	6.5	20.3	45.8	6.4	86.5	138.7
3	409	60	1710	2179	58	7	87	152	13.9	1.4	6.0	21.3	44.1	5.6	81.0	130.7
4	574	187	2236	2997	73	28	114	215	16.8	9.8	7.9	34.5	56.2	18.2	106.1	180.5
5	604	104	3608	3316	82	15	130	227	16.4	8.0	9.1	33.5	65.6	7.0	121.3	193.9
6	523	85	1590	2198	76	12	81	169	14.5	5.4	5.6	25.5	61.5	6.6	75.4	143.5
7	382	82	1762	2226	52	12	90	154	11.4	4.1	6.3	21.8	40.6	7.9	83.7	132.2
8	476	156	1754	2386	65	26	89	180	11.7	9.6	5.9	27.2	53.3	16.4	83.1	152.8
9	529	81	1928	2538	67	12	98	177	12.2	4.8	6.8	23.8	54.8	7.2	91.2	153.2
10	464	141	1025	1630	70	26	52	148	12.4	6.2	3.6	22.2	57.6	19.8	48.4	125.8
11	507	182	1755	2444	80	33	90	203	14.8	9.9	6.3	31.0	65.2	23.1	83.7	172.0
12	608	206	1626	2440	76	23	83	182	13.8	5.7	5.8	25.3	62.2	17.3	77.2	156.7
Total	6087	1457	22034	29578	848	224	1073	2145	166.5	74.4	74.4	315.3	681.5	149.6	998.6	1829.7
Av. per plant	507	121	1836	2466	70	19	89	179	13.9	6.2	6.2	26.3	56.8	12.8	82.8	152.6
Av. per sq. meter of leaf area	2013	480	7288	9790	292	75	353	711	55	25	25	104	227	50	328	607

\* Dry weight minus ash weight.

TABLE V  
GROWTH RESPONSE IN TERMS OF TOTAL PLANT PRODUCTION BY PLANTS SHADED WITH ONE LAYER OF CHEESECLOTH

PLANT	FRESH WEIGHT				DRY WEIGHT				ASH WEIGHT				PLANT FOOD WEIGHT*			
	Tops	Roots	Fruit	Total	Tops	Roots	Fruit	Total	Tops	Roots	Fruit	Total	Tops	Roots	Fruit	Total
1	gm. 465	gm. 144	gm. 854	gm. 1463	gm. 62	gm. 13	gm. 45	gm. 120	gm. 11.2	gm. 4.6	gm. 2.9	gm. 18.7	gm. 50.8	gm. 8.4	gm. 42.1	gm. 101.3
2	460	187	1048	1695	65	25	55	145	14.3	11.8	3.5	29.6	50.7	13.2	51.5	115.4
3	560	116	1495	2171	66	11	78	155	13.2	2.2	4.9	20.3	52.8	8.8	73.1	134.7
4	496	140	1397	2033	56	18	73	147	12.3	6.8	4.6	23.7	43.7	11.2	68.4	123.3
5	657	213	1037	1907	77	35	54	166	16.2	17.5	3.4	37.1	60.8	17.5	50.6	128.9
6	613	280	1009	1902	63	47	53	163	12.6	19.7	3.3	35.6	50.4	27.3	49.7	127.4
7	425	269	942	1636	54	49	50	153	11.8	15.7	3.1	30.6	42.2	33.3	46.9	122.4
8	521	179	873	1573	58	23	46	127	12.7	6.4	2.9	22.0	45.3	16.6	43.1	105.0
9	554	120	1805	2479	61	17	95	173	18.3	3.4	6.0	27.7	42.7	13.6	89.0	145.3
10	458	147	1132	1737	52	20	59	131	15.6	5.0	3.7	24.3	36.4	15.0	55.3	106.7
11	563	248	1709	2520	75	38	90	203	18.7	19.8	5.7	44.2	56.3	18.2	84.3	158.8
12	502	231	1308	2041	69	38	69	176	18.6	11.4	4.3	34.3	50.4	26.6	64.7	141.7
Total	6274	2274	14609	23157	758	334	767	1959	175.5	124.3	48.3	348.1	582.5	209.7	718.7	1510.9
Av. per plant	522	189	1217	1928	63	28	64	163	14.6	10.3	4.0	29.0	48.5	17.5	59.8	125.8
Av. per sq. meter of leaf area	1670	605	3894	6169	201	89	204	499	46	33	13	93	155	56	191	406

\* Dry weight minus ash weight.

TABLE VI  
GROWTH RESPONSE IN TERMS OF TOTAL PLANT PRODUCTION BY PLANTS SHADED WITH TWO LAYERS OF CHEESECLOTH

* PLANT	FRESH WEIGHT				DRY WEIGHT				ASH WEIGHT				PLANT FOOD WEIGHT*			
	ROOTS		FRUIT		ROOTS		FRUIT		ROOTS		FRUIT		ROOTS		FRUIT	
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
1	692	155	726	1573	79	21	40	140	21.3	4.2	2.4	27.9	16.8	37.6	112.1	112.1
2	414	132	885	1431	55	16	49	120	17.6	3.2	2.9	23.7	12.8	46.1	96.3	96.3
3	492	120	983	1595	54	15	54	123	11.3	2.7	3.3	17.3	12.3	50.7	105.7	105.7
4	480	199	945	1624	68	23	52	143	19.0	5.1	3.1	27.2	49.0	48.9	115.8	115.8
5	495	77	933	1505	64	9	51	124	15.4	1.6	3.1	20.1	48.6	7.4	47.9	103.9
6	476	142	1016	1634	60	18	56	134	18.0	4.5	3.4	25.9	42.0	52.6	108.1	108.1
7	540	124	878	1542	56	13	48	117	16.8	2.3	2.9	22.0	39.2	10.7	45.1	95.0
8	582	87	510	1179	69	12	28	109	13.8	2.1	1.7	17.6	55.2	26.3	91.4	91.4
9	616	91	892	1599	78	11	49	138	17.2	2.6	2.9	22.7	60.8	8.4	46.1	115.3
10	539	32	849	1420	62	5	47	114	13.0	1.2	2.8	17.0	49.0	3.8	44.2	97.0
11	434	68	1327	1829	57	7	73	137	13.7	1.4	4.4	19.5	43.3	5.6	68.6	117.5
12	554	35	875	1464	58	6	48	112	19.1	2.7	2.9	24.7	38.9	3.3	45.1	87.3
Total	6314	1262	10819	18395	760	156	595	1511	196.2	33.6	35.8	265.6	122.4	559.2	1245.4	1245.4
Av. per plant	526	105	902	1533	63	13	50	126	16.4	2.8	3.0	22.1	45.3	46.6	103.8	103.8
Av. per sq. meter of leaf area	1300	259	2230	3790	156	32	121	312	40	7	7	54	116	114	258	258

\* Dry weight minus ash weight.

TABLE VII  
LIGHT INTENSITY FOR PLANTS UNDER DIFFERENT TREATMENTS

MONTH	AV. DAILY LIGHT INTEN- SITY PER HOUR, IN AP- PARENT FOOT-CANDLES				TOTAL HOURS DAYLIGHT	TOTAL FOOT CANDLE HOURS*				PERCENTAGES NO SHADE = 100		
	NO SHADE	1 LAYER		2 LAYERS CHEESE- CLOTH		NO SHADE	1 LAYER CHEESE- CLOTH	2 LAYERS CHEESE- CLOTH	NO SHADE	1 LAYER CHEESE- CLOTH	2 LAYERS CHEESE- CLOTH	
		CHEESE- CLOTH	CHEESE- CLOTH									
Mar. 1-30 incl.	532	251	114		434	230888	108934	49476	100	52.1	21.4	
Apr. 1-30 incl.	980	497	212		420	411600	208740	89040	100	50.7	21.5	
May 1-31 incl.	1133	591	246		454	514382	268314	111684	100	51.9	21.7	
June 1-30 incl.	1872	973	468		459	859248	446607	214812	100	62.1	25.0	
July 1- 3 incl.	1774	892	355		46	81604	41032	16330	100	50.2	20.0	
Daily average during crop growth	1139.9	583.1	261.0									
Total for producing crop					1813	2,097,722	1,073,627	481,342				
* Daily average intensity × number of hours.												

\* Daily average intensity  $\times$  number of hours.

with close approximation, as the season advanced and ended. While the general intensity of the sunlight increased gradually for all, the three experimental conditions of full intensity, one-half intensity, and one-fourth intensity continued to hold and to be effective.

General correlation of these controlled variations in light intensity, previously shown with the differences in growth responses of the three lots of plants, is obvious. The relationship is itself negative in character for leaf area, and positive for fruit production and for total plant production. In order to facilitate inspection, certain figures from the preceding tables are brought together in table VIII.

TABLE VIII

RELATIONSHIP OF LIGHT INTENSITIES TO GROWTH RESPONSES OF THE PLANTS

LIGHT INTENSITY	TOTAL FOOT CANDLE HOURS OF LIGHT (TABLE VI)	AV. LEAF AREA PER PLANT (TABLE I)	AVERAGE HEIGHT OF PLANT (TABLE II)	TOTAL WEIGHT OF FRUIT (TABLE III)	TOTAL FRESH WEIGHT OF PLANTS (TABLES IV, V, VI)
		<i>sq. cm.</i>	<i>cm.</i>	<i>gm</i>	<i>gm.</i>
Unshaded	2097722	4385	81	22034	29578
1 layer cheese- cloth	1073627	5559	102	14607	23157
2 layers cheese- cloth	481342	7072	117	10819	18395

However, the association which is apparent in table VIII can have validity only in case certain other factors, which are known to condition the photosynthetic rate and, consequently, growth, remained sufficiently constant during the experimental period. Table IX gives data respecting three such factors—these being the principal ones which required consideration and control.

These three factors were relatively uniform for each block of plants. Their variations in the three treatments is regulated somewhat by the light intensity. Thus, light intensity variation mainly accounted for the plant growth responses under each condition.

## DAILY PERIODS OF MEASUREMENT

The data which have been presented show, beyond doubt, a relationship between the behavior of the plants and the different light intensities under which they grew and matured. The evidence, however, is general in nature and not such as to be adequate for those mathematical processes which yield quantitative expressions of correlation.

TABLE IX

AIR TEMPERATURE, SOIL TEMPERATURE, AND RELATIVE HUMIDITY DURING GROWTH PERIOD

MONTH	AIR TEMPERATURE								
	NO SHADE			1 LAYER CHEESECLOTH			2 LAYERS CHEESECLOTH		
	MAX.	MIN.	24-HR. AV.	MAX.	MIN.	24-HR. AV.	MAX.	MIN.	24-HR. AV.
March	°F. 73	°F. 56	°F. 62	°F. 82	°F. 58	°F. 66	°F. 80	°F. 58	°F. 67
April	88	55	65	86	55	66	86	56	66
May	86	85	66	90	55	67	90	55	66
June	98	46	73	95	48	71	95	48	71
July	106	55	76	100	54	73	102	56	70

MONTH	SOIL TEMPERATURE								
	NO SHADE			1 LAYER CHEESECLOTH			2 LAYERS CHEESECLOTH		
	MAX.	MIN.	24-HR. AV.	MAX.	MIN.	24-HR. AV.	MAX.	MIN.	24-HR. AV.
March	°F. 62	°F. 62	°F. 62	°F. 66	°F. 61	°F. 63	°F. 72	°F. 60	°F. 64
April	63	60	62	67	60	64	70	60	66
May	63	60	62	69	60	63	74	60	66
June	65	58	62	76	59	66	82	53	74
July	67	59	63	77	60	71	82	65	74

MONTH	RELATIVE HUMIDITY								
	NO SHADE			1 LAYER CHEESECLOTH			2 LAYERS CHEESECLOTH		
	MAX.	MIN.	24-HR. AV.	MAX.	MIN.	24-HR. AV.	MAX.	MIN.	24-HR. AV.
March	°F. 76	°F. 40	°F. 61	°F. 80	°F. 41	°F. 65	°F. 80	°F. 41	°F. 62
April	83	32	64	84	38	73	84	38	65
May	84	27	68	90	42	79	84	30	72
June	84	30	68	90	34	75	84	30	73
July	91	28	63	85	30	72	84	30	70

The plant materials manufactured by the plants on twelve dates, spread over the period of growth, were determined on seven periods during each of the days. This procedure for estimating the photosynthetic activity was that termed the "Modified Sachs Method." It consisted of taking 2 sq. cm. of foliage from each plant every 2 hours in sample bottles and weighing in the fresh condition. These small discs of the leaves were then heated in the oven

at 70° C. for 12 hours and then at 95° C. for 6 hours, when they were reweighed. After drying they were put in crucibles and ashed, and again reweighed. The dry weight minus the ash weight was the amount of photosynthesized product in each sample. Correction for respiration and translocation was made by adding the average loss in weight per 2 hours during the night to the difference in weight of the 2-hour samples during the day.

Light in each block of plants was measured at 2-hour intervals with a Clement's photometer in which *solio* paper is used, and comparisons made with a standard. On days when photosynthetic activity was determined, the Macbeth illuminometer was also used in order to get readings in actual number of foot-candles. The data are presented in tables X, XI, and XII.

TABLE X  
PHOTOSYNTHATE AND LIGHT INTENSITIES FOR UNSHADED PLANTS

DATE	VARIABLES	PERIOD OF DAY						
		4-8 A.M.	8-10 A.M.	10-12 A.M.	12-2 P.M.	2-4 P.M.	4-6 P.M.	6-8 P.M.
April 7 .....	Photosynthate	1.12	1.11	1.60	2.96	5.84	2.44	0.36
	Light intensity	606	1200	1411	2408	2314	1207	117
April 24 .....	Photosynthate	1.60	1.46	0.16	2.16	4.20	1.40	1.64
	Light intensity	200	400	444	387	256	139	87
April 29 .....	Photosynthate	1.64	3.06	3.06	4.08	1.24	2.28	1.60
	Light intensity	732	1464	2553	2553	1445	806	337
May 5 .....	Photosynthate	1.48	1.46	1.44	1.60	1.76	0.56	0.27
	Light intensity	212	424	393	394	287	116	87
May 13 .....	Photosynthate	1.06	1.06	1.44	2.64	0.88	0.72	0.34
	Light intensity	237	446	286	1285	2408	2408	337
May 14 .....	Photosynthate	1.28	1.40	2.00	3.96	1.68	1.80	0.36
	Light intensity	1164	2078	2409	2503	1800	687	140
May 23 .....	Photosynthate	2.48	1.80	0.76	1.84	7.84	1.24	1.68
	Light intensity	1206	2172	2588	2937	2730	1376	736
May 24 .....	Photosynthate	1.52	3.20	4.96	1.32	2.92	1.76	1.60
	Light intensity	637	1230	2499	2435	2100	1350	736
June 9 .....	Photosynthate	3.76	3.32	2.76	6.04	3.00	2.92	1.96
	Light intensity	739	1369	2533	2855	2344	964	674
June 15 .....	Photosynthate	1.84	1.40	2.76	4.88	2.96	2.76	2.16
	Light intensity	100	186	277	278	944	910	121
June 16 .....	Photosynthate	1.88	2.76	5.44	7.20	5.12	1.60	1.40
	Light intensity	210	330	3480	3906	3773	3039	1674
June 17 .....	Photosynthate	4.03	2.64	1.52	9.20	5.12	1.60	1.08
	Light intensity	940	1734	1503	3155	4235	3115	1769
Av. photosynthate .....		582	1086	1520	2091	2053	1426	578
Light intensity .....		1.89	2.05	2.32	3.99	3.54	1.75	1.20

**TABLE XI**  
**PHOTOSYNTHATE AND LIGHT INTENSITIES FOR PLANTS SHADED WITH ONE LAYER**  
**OF CHEESECLOTH**

DATE	VARIABLES	PERIOD OF DAY						
		4-8 A.M.	8-10 A.M.	10-12 A.M.	12-2 P.M.	2-4 P.M.	4-6 P.M.	6-8 P.M.
April 7	Photosynthate	1.00	0.76	0.16	0.28	3.44	1.44	0.36
	Light intensity	100	202	246	236	128	49	24
April 24	Photosynthate	1.00	1.06	1.36	1.28	2.16	2.80	0.80
	Light intensity	337	648	768	1245	1135	544	49
April 29	Photosynthate	0.92	1.60	1.72	1.84	1.84	2.00	0.60
	Light intensity	462	739	1182	1281	813	739	247
May 5	Photosynthate	1.42	0.88	1.60	1.04	1.60	0.12	0.16
	Light intensity	104	241	187	187	136	83	64
May 13	Photosynthate	0.80	0.60	1.80	1.32	0.52	0.24	0.28
	Light intensity	106	207	182	677	1163	1231	187
May 14	Photosynthate	1.00	1.64	1.60	1.16	1.72	0.88	0.16
	Light intensity	737	1207	1251	1409	914	352	47
May 23	Photosynthate	1.68	1.48	0.16	1.28	1.32	1.12	0.04
	Light intensity	737	894	1191	1113	1113	689	306
May 24	Photosynthate	1.68	2.24	1.48	1.04	1.52	0.96	0.40
	Light intensity	331	532	1170	1180	1259	532	306
June 9	Photosynthate	0.76	0.12	1.16	0.32	1.20	2.48	0.16
	Light intensity	312	692	1069	1424	1177	409	394
June 15	Photosynthate	0.40	1.06	1.40	2.36	1.28	2.24	0.04
	Light intensity	100	153	128	157	476	451	86
June 16	Photosynthate	1.46	2.74	0.12	2.64	2.40	1.28	0.40
	Light intensity	96	823	1793	1946	1891	1562	815
June 17	Photosynthate	4.12	2.28	2.80	4.20	4.96	2.08	0.18
	Light intensity	431	586	796	1025	2124	1515	815
Av. photosynthate		1.35	1.37	1.28	1.56	1.91	1.47	0.29
Light intensity		321	577	830	990	1024	679	279

It is shown by tables X, XI, and XII that with the unshaded plants a greater amount of light is necessary for each gram of photosynthate manufactured. Furthermore, the amounts of plant food appear to increase until 12-2 P.M. when the light intensity reaches its maximum and then decreases at a relatively similar rate with the light. Differing from this, the plants shaded show a slower increase in food manufacture relative to the light increase until the 2-4 P.M. period, when they reach the maximum, and then they decrease more rapidly in ratio with the light intensity. Greater reduction in light shows a more gradual increase in photosynthesis and there appears to be an accumulation of plant food over a longer period, or a lagging in



**TABLE XII**  
**PHOTOSYNTHATE AND LIGHT INTENSITIES FOR PLANTS SHADED WITH TWO LAYERS**  
**OF CHEESECLOTH**

DATE	VARIABLES	PERIOD OF DAY						
		4-8 A.M.	8-10 A.M.	10-12 A.M.	12-2 P.M.	2-4 P.M.	4-6 P.M.	6-8 P.M.
April 7 .....	Photosynthate	0.52	0.44	1.12	.00	2.08	1.24	0.32
	Light intensity	87	92	104	148	96	45	16
April 24 .....	Photosynthate	0.20	0.44	0.72	1.24	2.60	2.68	0.40
	Light intensity	92	341	354	684	578	257	26
April 29 .....	Photosynthate	0.20	1.32	1.68	1.32	1.20	1.68	0.40
	Light intensity	141	371	600	661	407	303	124
May 5 .....	Photosynthate	0.16	0.12	0.60	1.16	1.24	1.00	0.32
	Light intensity	109	116	101	75	72	37	24
May 13 .....	Photosynthate	0.46	0.32	0.36	0.76	0.24	0.24	0.28
	Light intensity	83	123	99	384	561	647	90
May 14 .....	Photosynthate	0.36	0.20	0.64	0.72	0.44	0.56	0.26
	Light intensity	361	683	630	754	456	106	24
May 23 .....	Photosynthate	0.68	1.56	0.28	1.08	2.80	1.44	0.40
	Light intensity	337	491	573	602	520	297	152
May 24 .....	Photosynthate	1.12	0.96	1.28	0.72	2.76	1.04	0.32
	Light intensity	113	225	552	519	635	225	147
June 9 .....	Photosynthate	0.60	0.06	0.16	0.84	0.88	1.48	0.56
	Light intensity	185	386	548	760	680	230	155
June 15 .....	Photosynthate	0.20	0.86	0.40	3.20	1.40	0.72	0.28
	Light intensity	40	90	91	85	238	213	107
June 16 .....	Photosynthate	0.60	0.37	0.20	3.24	1.36	1.36	0.16
	Light intensity	40	490	937	951	946	744	521
June 17 .....	Photosynthate	4.20	2.52	1.04	1.83	4.04	1.32	0.10
	Light intensity	261	276	467	524	1080	784	521
Av. photosynthate .....		0.77	0.76	0.70	1.34	1.75	1.23	0.32
Light intensity .....		154	307	421	512	522	324	158

photosynthate manufacture, when the light is decreased due to heavy shading. This appears to result in the plants exposed only to light of low intensities having a much lower basal metabolism than no-shade plants. The simple coefficients of correlation for photosynthate and light,  $r = 0.5454 \pm 0.0527$ ,  $r = 0.3012 \pm 0.0681$ , and  $r = 0.3034 \pm 0.0679$ , demonstrate the importance of the light to the plant food manufacture under the no-shade condition as compared with shaded plants.

While temperature and humidity are similar or relatively uniform for the three lots of plants, it varied the same for each, as the day advanced. Naturally, this would be expected to be true, owing to their relationship to

light intensity and its variation. Consequently, data on temperature and humidity were taken for each of the 2-hour periods. The simple coefficients of correlation for photosynthate and temperature,  $r = 0.2968 \pm 0.0681$ ,  $r = 0.1924 \pm 0.0725$ , and  $r = 0.1704 \pm 0.0727$ , signify that the temperature is in close relation with the light intensity and probably is intercorrelated with it. Their being lower than those for light demonstrates their slighter importance in photosynthetic activity.

The simple correlation coefficients for photosynthate and humidity,  $r = -0.2099 \pm 0.0714$ ,  $r = -0.4955 \pm 0.0565$ , and  $r = -0.3377 \pm 0.0663$ , indicate that humidity is possibly too high for proper plant food manufacture. Their negative character signifies that the high humidity may have a tendency to hinder photosynthesis and the higher the negative correlation the greater it is reduced. This appears to be one of the contributing causes of lower plant food manufacture when the plants are shaded.

#### CORRELATION COEFFICIENTS

In order to measure the direct effect of light intensity on photosynthesis, it is necessary to know how much the other environmental factors affect photosynthesis, and the relation between all of these factors. It appears that the true value of this relationship cannot be obtained directly from the raw figures, but an analysis of the data must be completed in order to determine the numerical measurements. This analysis will show the relative importance of the variation of each of these independent variables on the variation in the dependent variable and can best be demonstrated by the correlation coefficients given in table XIII.

NO-SHADE PLANTS.—The zero order coefficients seem to show a much greater relationship between  $X_2$  and  $X_1$  than between  $X_1$  and  $X_1$  or  $X_4$  and  $X_1$ . The relationships between  $X_1$  and  $X_1$ , and  $X_4$  and  $X_1$  are questionable because of the possibility of their being obscured by the relationships between the independent variables. Because of this we have separated the effects of the independent variable in order to get the first order coefficients. This separation tends to confirm the tentative conclusion reached with the zero order coefficients (that the major relationship is that between  $X_1$  and  $X_2$ ). The conclusions appear to be still slightly questionable because only two of the independent variables have been considered at a time. Because of this we shift to the second order coefficients. This demonstrates that when we consider the effect of variation in light intensity alone (both temperature and humidity being constant), we can explain 27.7 per cent. of the variation in photosynthate, while variation in temperature explains but 1 per cent. and variation in humidity explains but 4 per cent. of the photosynthate variation. The coefficient of multiple correlation shows that the three factors taken together explain 32.4 per cent. of the photosynthate

TABLE XIII

CORRELATION COEFFICIENTS FOR PHOTOSYNTHATE AND ENVIRONMENT\*

r (0 ORDER)				r (1ST ORDER)				r (2ND ORDER)				R (MULTIPLE)			
SUB-SCRIPT	COEFFICIENT			SUB-SCRIPT	COEFFICIENT			SUB-SCRIPT	COEFFICIENT			SUB-SCRIPT	COEFFICIENT		
	No SHADE	1 LAYER CHEESE-CLOTH	2 LAYERS CHEESE-CLOTH		No SHADE	1 LAYER CHEESE CLOTH	2 LAYERS CHEESE CLOTH		No SHADE	1 LAYER CHEESE-CLOTH	2 LAYERS CHEESE-CLOTH		No SHADE	1 LAYER CHEESE-CLOTH	2 LAYERS CHEESE-CLOTH
12	0.5454 ±0.0527	0.3012 ±0.0681	0.3034 ±0.0679	12.3	0.4872 ±0.0571	0.2421 ±0.0309	0.2600 ±0.0698								
				12.4	0.5421 ±0.0528	-0.0372 ±0.0748	0.1595 ±0.0728	12.34	0.5266 ±0.0541	0.0236 ±0.0747	0.1711 ±0.0727				
13	0.2968 ±0.0681	0.1924 ±0.0721	0.1704 ±0.0727	13.2	0.0009 ±0.0749	-0.0049 ±0.0749	-0.0087 ±0.0749								
				13.4	0.2203 ±0.0716	0.01436 ±0.0733	0.0173 ±0.0748	13.24	0.1025 ±0.0741	-0.1452 ±0.0733	0.0649 ±0.0745				
14	-0.2099 ±0.0714	-0.4955 ±0.0565	-0.3377 ±0.0663	14.2	0.1716 ±0.0727	-0.4179 ±0.0618	-0.2235 ±0.0717								
				14.3	-0.0197 ±0.0748	-0.4927 ±0.0567	-0.3002 ±0.0678	14.23	0.2021 ±0.0718	-0.4457 ±0.0600	-0.2348 ±0.0708	1.234	0.5695 ±0.0505	0.5108 ±0.0553	0.3731 ±0.0644

\*  $X_1$  = photosynthate,  $X_2$  = light intensity,  $X_3$  = temperature,  $X_4$  = humidity.

variation. Light intensity alone, as we have seen, accounts for 27.7 per cent. showing that temperature and humidity are negligible factors in photosynthesis except where they are correlated with light intensity.

**ONE-LAYER-OF-CHEESECLOTH PLANTS.**—It appears that the humidity is too high for proper use of light by the plant in photosynthesis. The humidity is consistently higher in this block of plants than in the other blocks (table IX). This may account for the reduction in photosynthate as compared with the no-shade block of plants. The correlation coefficients demonstrate this fact in every case.

**TWO-LAYERS-OF-CHEESECLOTH PLANTS.**—The humidity is evidently too high for the plants to utilize light to the best advantage. It appears that light intensity is possibly too low, even at the best, for proper food manufacture. The total effect of light, temperature, and humidity in this block of plants explained only 13.9 per cent. of the photosynthate variation which is about one-half that of the no-shade plants. This demonstrates that some other factors, that were not taken into consideration, probably have a definite effect on the photosynthetic activity.

#### SUPPLEMENTARY CONSIDERATIONS

**CHLOROPHYLL CONTENT.**—The relation of radiation to pigmentation is of very great importance owing to the necessity of light for the formation of pigments, and to the fact that the pigments absorb radiant energy which is essential for the photosynthetic activity of the plants. The naturally occurring plant pigments, which are found in the cell structure of the plant foliage, are chlorophyll, carotene, and xanthophyll. These pigments per unit leaf area were determined for each group of plants, at several periods during the experiment. The modified WILLSTÄTTER and STOLL method of extraction (22) was used, and the comparison against a standard made with the DuBoscq colorimeter. The data are given in table XIV.

It appears from table XIV that light intensity is effectual for proper chromogenesis in the tomato foliage. According to expectations, the no-shade plants contained more chlorophyll per square centimeter of leaf area, and the shaded plants showed a variation according to the amount of light the plants received. The reduction in photosynthate manufacture due to shading was relatively proportional to the amount of chlorophyll per square centimeter of leaf area. Although this reduction did not affect the chlorophyll efficiency, it did affect the total plant food manufacture.

**LEAF ANATOMY.**—Histological sections were made, at several different times during the experimental period, of the leaves of the plants under test. Only leaves strictly comparable in position, degree of maturity, and so forth, were used. The result is given in figure 1, for the sections obtained on June 17.

TABLE XIV  
FOLIAGE PIGMENTS

	NO SHADE	1 LAYER CHEESE- CLOTH	2 LAYERS CHEESE- CLOTH
Chlorophyll—Mg. per sq. cm.			
April 15 .....	0.0368	0.0255	0.0189
April 15 .....	0.0379	0.0257	0.0189
May 10 .....	0.0477	0.0353	0.0114
May 10 .....	0.0468	0.0377	0.0125
June 17 .....	0.0474	0.0309	0.0110
June 17 .....	0.0487	0.0392	0.0177
June 21 .....	0.0401	0.0302	0.0187
June 21 .....	0.0391	0.0300	0.0186
Total .....	0.3455	0.2545	0.1277
Average .....	0.0432	0.0318	0.0159
Xanthophyll—Mg. per sq. cm.			
April 15 .....	0.0024		
April 15 .....	0.0028		
May 10 .....	0.0020	0.0015	0.0008
May 10 .....	0.0024	0.0014	0.0008
June 17 .....			
June 17 .....			
June 21 .....	0.0028	0.0017	0.0010
June 21 .....	0.0028	0.0014	0.0012
Total .....	0.0152	0.0060	0.0038
Average .....	0.0025	0.0015	0.0009
Carotene—Mg. per sq. cm.			
April 15 .....	0.0014	0.0014	0.0010
April 15 .....	0.0017	0.0012	0.0010
May 10 .....	0.0014	0.0011	0.0006
May 10 .....	0.0011	0.0011	0.0006
June 17 .....			
June 17 .....			
June 21 .....	0.0013	0.0011	0.0006
June 21 .....	0.0012	0.0010	0.0006
Total .....	0.0081	0.0069	0.0044
Average .....	0.0013	0.0011	0.0007

In general, the cell development of the leaves under the no-shade condition is normal, but when shaded the cells in the spongy parenchyma lack regularity in shape and are arranged loosely, so that a large part of their surface is exposed to the intercellular spaces. The greater the shade, the less the palisade parenchyma cells are developed. This demonstrates how the number of palisade layers and the density of the cell structure depends largely, either directly or indirectly, upon light intensity.

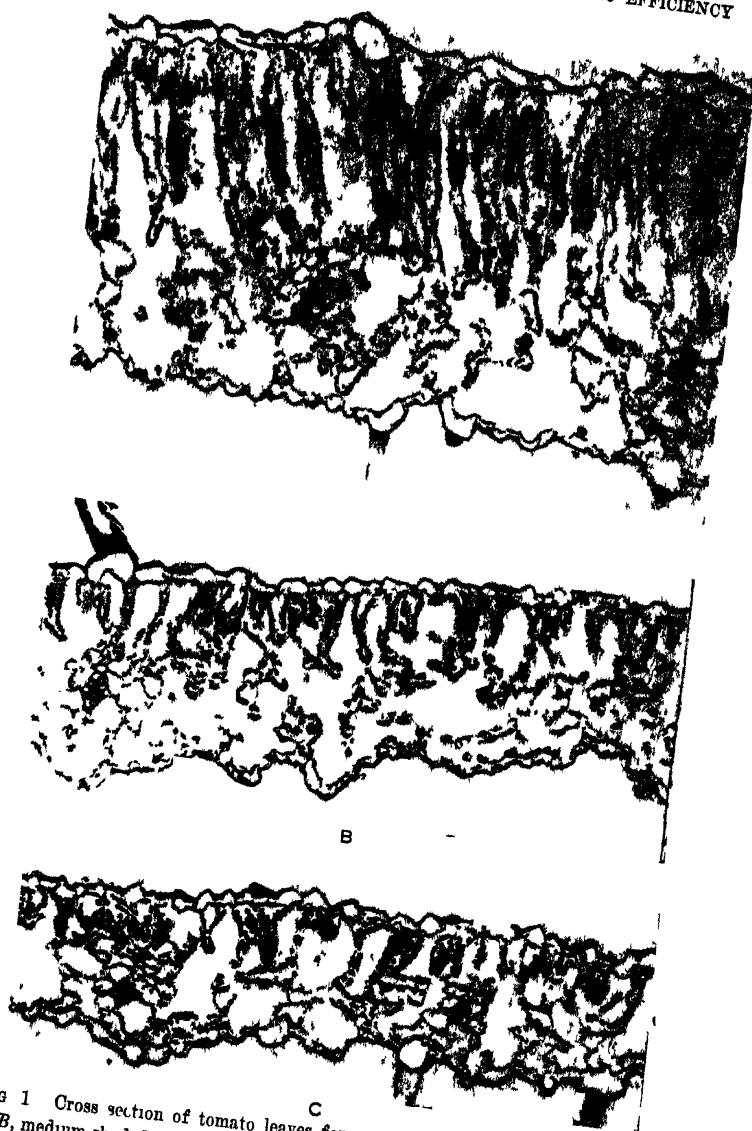


FIG 1 Cross section of tomato leaves for specimens taken June 17 A, unshaded plant, B, medium shaded plant, C heavily shaded plant All  $\times 250$

These supplementary factors are rather definitely regulated by the amount of light received by the plants, and this appears to be in order with the variation of photosynthetic activity When the plants are exposed to

the no-shade condition the palisade cells are well-developed and their chloroplasts seem to arrange themselves so as to decrease the surface and transpiration due to the light, but when shaded they are differently arranged and thereby increase the surface for receiving light. This latter arrangement appears to increase the chloroplast's efficiency, and the greater the light reduction the more it is increased. It appears that the reduction in photosynthetic rate did not have an effect on the chloroplast efficiency. As previously stated, the light seems to have a regulatory effect on the chloroplast content and cell structure of the leaves, and this is one of the contributing causes for a decreased photosynthetic activity by the plants when shaded.<sup>3</sup>

### Discussion

This study has dealt primarily with the influence of light intensity on photosynthetic activity of tomato plant leaves, as measured by amount of growth, fruit production, and increases in fresh and dry weights. The results show, as expected, that, on the whole, there is a close relationship between these several factors; *viz.*, with decreased light intensity there is (a) greater vegetative growth, as measured by leaf area, and both fresh and dry weight of tops and roots, (b) decreased fruit production, and (c) a decrease in the total amount of photosynthate produced by the plants. However, the increase in vegetative growth and the decreases in fruit production and total photosynthate produced are not directly proportional to the decreases in light intensity. Thus, reducing light intensity by one-half resulted in only approximately a one-fourth increase in amount of vegetative growth, a one-third decrease in fruit production, and a one-sixth decrease in total photosynthate production. Reducing light intensity to one-fourth normal resulted in only a 40 per cent. increase in vegetative growth, a one-half decrease in fruit production and a one-third decrease in total photosynthate production (table XV). This is but another way of saying that the partially shaded leaves used their limited supply of light more efficiently than the unshaded leaves used their normal supply. That is, a given quantity of light affected a greater photosynthate production in the case of the shaded plants than was true in the case of those unshaded.

Great, however, as were the differences between the growth rates, leaf areas, and fruit and photosynthate production of the several groups of plants exposed to the different light intensities, there were equally great differences between different plants within the same group, in their apparent ability to utilize their light supply for fruit and photosynthate production. Indeed, some of the individuals (no. 11) in the moderately shaded

<sup>3</sup> The cells of the upper part of the thick leaf in the no-shade group removes enough red and violet light rays to reduce the effectiveness on the lower leaf cells, but this blocking effect is not so apparent in the thin-shaded leaves.

TABLE XV  
PLANT EFFICIENCY

	SHADING	NO. FRUITS PER PLANT	FRESH WEIGHT OF FRUIT PER PLANT	LEAF AREA PER PLANT	PLANT FOOD WEIGHT PER PLANT	LEAF AREA NECESSARY FOR PRODUCING EACH GRAM OF PLANT FOOD PER PLANT	TOTAL FOOT- CANDLE HOURS OF LIGHT RE- CEIVED DURING SEASON (INTENSITY X HOURS)
Table IV Plant 5	No shade	32	2608	sq. cm. 5436	gm. 193.9	sq. cm. 28.0	2,097,722
Plant 10	No shade	11	1025	4176	125.8	33.2	"
Average for 12 plants	No shade	22.5	1836	4563	152.6	29.2	"
Table V Plant 1	1 layer cheesecloth	9	854	5115	101.3	50.5	1,073,627
Plant 11	1 layer cheesecloth	22	1709	6193	158.8	38.9	"
Average for 12 plants	1 layer cheesecloth	17.0	1217	5559	125.8	44.1	"
Table VI Plant 11	2 layers cheesecloth	14	1327	5642	117.5	48.1	481,342
Plant 12	2 layers cheesecloth	9	875	7566	87.3	86.6	"
Average for 12 plants	2 layers cheesecloth	11.8	902	7072	103.8	68.2	"



group produced nearly as much photosynthate per unit of leaf as some of those in the unshaded group and one of those in the heavily shaded group (no. 11) produced nearly as much photosynthate per unit of leaf area as the average of those in the moderately shaded group and within 30 per cent. as much as some of the least efficient in the unshaded group (table XV).

This latter fact is of special significance for it suggests the possibility of developing a strain of plants that has a high degree of photosynthetic efficiency under conditions of low light intensity. Obviously, the producer of indoor-grown tomatoes has no control over light intensity—at least he has no practicable means of increasing it. However, if he can obtain a varietal selection which is especially adapted to the low light intensities and short days of the northern winter season, a substantial contribution will have been made to the solution of the problem of profitably growing tomatoes in the greenhouse during the winter. Little or no effort has thus far been directed toward developing such a physiological strain of tomatoes, present stocks apparently being heterozygous in this respect. The studies here reported point clearly to some of the possibilities that lie in this direction.

### Summary

The effect of light intensity on the photosynthetic efficiency of tomato plants was studied by growing Grand Rapids Forcing tomato plants under three different daily average light intensities, 1139.9, 583.1, and 261.0 foot-candles. The results were as follows:

1. The responses in stem elongation and leaf-area expansion were both continuously and finally greater when the light intensity was reduced, showing a negative relationship.

2. It was indicated that when the light intensity reached a definite average the fruit would set rather freely and develop.

3. The percentages of dry matter, ash material, water, fresh weight, and elaborated food materials correlate rather closely with the light intensity received by the plants. Light intensity variation is the chief cause of differences in plant efficiency.

4. Basal plant metabolism and its contributing factors are regulated by the amount of light received by the plants.

5. The increase in the multiple correlations (when the elaborated food materials are the dependent variable, and light intensity, humidity, and temperature are the independent variables) over the simple correlations under each degree of light intensity is evidence that there is interrelation between factors regulating the plant food manufacture. The coefficients of determination demonstrate that light intensity alone accounts for 32.4 per cent. of the photosynthate variation and that temperature and humidity are negligible factors only when correlated with light intensity—humidity becom-

ing a critical factor in photosynthesis when the light intensity is reduced.

6. The light intensity appeared to have a regulatory effect on the average amounts of chlorophyll per square meter of leaf area. The chloroplasts in the leaves arranged themselves so as to get the greatest amount of light when it was reduced.

7. The leaf anatomy shows abnormal cell development when the plants are shaded. This abnormality consists of loosely arranged, irregular spongy parenchyma cells and a reduction in size, density, and number of palisade cells.

8. It is evident that light intensity averaging 1139.9 foot-candles daily during the growth of the tomato plants had a greater effect in promoting chlorophyll formation, fruit production, and photosynthetic efficiency than light of a daily average of 583.1 foot-candles, and this, in turn, had a similar greater effect than that on the plants receiving a daily average light intensity of 261.0 foot-candles.

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# EFFECT OF CERTAIN NUTRIENT DEFICIENCIES ON STOMATAL BEHAVIOR

MANHER C. DESAI

(WITH TEN FIGURES)

## Introduction

Although a great deal of research has been done on stomata, nevertheless, the facts regarding these important plant structures are still fragmentary and there are differences of opinion as to their fundamental characteristics. In 1895 BLACKMAN (3) demonstrated the importance of stomata in gaseous exchange, although the possible relations of stomata to transpiration had long been recognized. Regarding the regulatory rôle of stomata in respect to transpiration, differences of opinion exist. That the stomata open and close periodically during twenty-four hours has been established. Various theories have been advanced concerning the mechanism of opening and closure and the cellular changes associated with these. According to some, the changes of starch to sugar and *vice versa* with consequent changes in osmotic relations are responsible for the opening and closure. SCARTH (31), in recent years, has emphasized the importance of changes in the pH of the guard-cell vacuome due to changes in carbon dioxide content of the leaf, the opening and closure of the stomata being dependent directly or otherwise upon the effects of pH on starch-sugar changes or protein hydration, or both.

Whatever eventually may be found to be the actual mechanism involved in the opening and closure of stomata, it is clear that the metabolism of the guard cells and the adjacent tissues must also be involved. While much attention has been given to the direct influence of light, of leaf water content, and of chemical reagents and reactions on stomatal behavior, no attention, except for the work of PLEASANTS (27), has been given to the part played by nutrients on stomatal behavior. There has been some work reported on the effect of nutrient deficiency on the rate of photosynthesis and respiration by GRUZIT and HIBBARD (11), BRIGGS (4), LYON (20, 21), GREGORY and RICHARDS (8), and others.

Since stomatal behavior through its effects on the loss of water and gaseous exchange may markedly influence plant behavior, a fuller knowledge of the effect of various factors on stomatal behavior is desirable. Of the factors that are of importance, theoretically and practically, that of mineral nutrient supply may be of considerable significance.

## Materials and methods

### MATERIAL

In order to find whether stomatal behavior is influenced by mineral nutrition, various types of plants were grown in culture media deficient in potassium, phosphorus, or nitrogen. Some of the plants included in the experiments were maize (*Zea mays*), *Tradescantia* sp., Canada field peas (*Pisum sativum*), red kidney beans (*Phaseolus vulgaris*), and tobacco (*Nicotiana tabacum*).

These plants represent two distinct structural types of stomata found among the angiosperms, (1) the grass type and (2) the ordinary type. The ordinary type can be subdivided, on the basis of arrangement, into two classes: (a) regular, and (b) scattered.

The stomata of the regular type show a tendency towards formation of rows with the long axis of each stoma practically parallel to the long axis of the leaf, while the accessory cells, which are often present, are like other epidermal cells, except that they are generally a little smaller. The scattered stomata have no tendency towards formation of rows and there is no plan in the orientation of their axes, which lie at any angle to the long axis of the leaf. Many have no definite accessory cells. *Tradescantia*, some of the orchids (Orchidaceae), and certain other plants have the regular type, while *Pisum*, *Phaseolus*, *Nicotiana*, and many other dicotyledonous plants have the scattered type.

In all the experiments care has been taken to use functioning leaves. The first two leaves near the growing point are decidedly immature, while those near the basal end of the shoot are quite old, as shown by their yellowing and dying. In these cases, the stomatal behavior will not be normal. On the other hand, the third and fourth leaves have been found to be active and mature. The growth curve for the leaf tends to flatten when the leaf comes to be the third or fourth from the tip of the stem. Dr. PARRIS (oral communication) has found that at this stage the assimilation rate of *Phaseolus* reaches a maximum. These leaves, being neither too young nor too old, were used for the work reported here.

With *Phaseolus*, two opposite leaflets of a compound leaf were used, while in *Pisum* the first pairs of leaflets, counting from the stipules, were used. At least two plants were taken for each observation. These were so placed that the third or fourth leaf of each plant had a uniform light exposure.

At the time of observation, the readings were taken from near the tip of the leaf. In *Zea*, *Tradescantia* sp., and in most monocotyledonous plants, the first part of the leaf to emerge from the sheath is the tip, the base being the last. In these cases the use of the leaf tip prevents any accidental use of immature stomata.

With the dicotyledonous plants used, observations showed that the region of the tip generally stops growth first, the basal region continuing growth the longest. The work by AVERY (2) and others on tobacco shows this to be true.

Preliminary experiments with *Pisum* and other plants showed that the stomata from the tip region are much more sensitive to slight changes in light, leaf water content, and other environmental factors. This is especially true in plants with large leaves.

#### METHODS OF STOMATAL STUDY

One method for the study of stomatal behavior is rarely suited to all plants. The several methods available may be grouped as (1) direct and (2) indirect.

At the beginning of this work, it was decided to test all the available methods and to adopt the most suitable ones. MAXIMOV (23) has discussed some of the features of these methods in detail, and his work should be consulted for some of the features omitted.

Three indirect methods in general use for the study of stomatal behavior are (a) the MOLISCH injection method, (b) the porometer method, and (c) the hygrometric methods. By the MOLISCH method a qualitative reading can be obtained. The method, though quite useful and rapid under field conditions, could not be used to advantage in comparative studies where stomatal measurements differ often by not more than a micron or so. Various investigators have tried to make it a quantitative method. My own attempts to do so have so far given no satisfactory results.

The porometer method, although having certain disadvantages, has distinct advantages of its own when used with care and discretion. The important sources of error have been studied in detail by KNIGHT (13). This method has at least two disadvantages in a critical study. It measures resistance to a pressure flow, which is different from a resistance to diffusion, which is the normal method of gas movement. If what ASHBY (1) found is true, it also shows a flow of 15 to 26 per cent. when the pores *appear* to be completely closed. Furthermore, ASHBY's pressure flow measurements were subject to temperature changes, and he makes no attempt to correct for this when comparing with pore size.

The most important use for this method is to get correlative data on four distinct plant processes every hour, or possibly oftener. These processes are stomatal behavior, transpiration, carbon dioxide assimilation, and respiration. No use of this method has been made for such purposes except by MASKELL (22), who measured the carbon dioxide assimilation and stomatal opening at the same time. Nobody has used this method to get correlative data on all four of these processes.

Because of the lack of sensitiveness of this method, when the stomata are mostly closed, and also because the changes in the size of intercellular spaces offer varying resistances, this method was not suitable for the present work.

The hygrometric methods got impetus from F. DARWIN (*cf.*, MAXIMOV'S monograph), who assumed that transpiration was proportional to the stomatal opening. When it was shown that such an assumption was not strictly true, the hygrometric methods came into general disuse except occasionally by some authors in measuring stomatal opening.

The best hygrometric method is the cobalt chloride paper method. It has been used extensively in measurement of transpiration. Here the data for transpiration do not give absolute transpiration but transpiration under certain standard conditions. The application of cobalt chloride paper, as is done in the most improved form of this method, causes all the water vapor issuing from the leaf to be absorbed rather quickly. The result is that the gradient of diffusion of water vapor becomes much steeper than it was before the application of the cobalt chloride paper. This diffusion gradient will remain approximately constant, except when the leaf is near wilting or just before the color change takes place. Here the rate of movement of water vapor will be conditioned only by the stomatal aperture if we assume that the humidity in the substomatal chambers remains constant and if the cuticular transpiration is not great. Then the speed of color change will denote roughly the size of the stomatal aperture.

This method and the porometer method have a certain resemblance, since each measures the movement of gas. The cobalt chloride method differs from the porometer method in that it measures the diffusion of water vapor under standard conditions instead of the flow of gases under diminished pressure. But the varying resistance offered by the intercellular spaces may play a rôle in limiting the usefulness of this as well as the porometer method. If the intercellular spaces at certain times offer greater resistance to diffusion of water vapor than does the stomatal aperture we may not measure the aperture at that particular time but the rate of diffusion conditioned by the intercellular spaces. How important a rôle the intercellular resistance plays in conditioning the diffusion of gases in and out of a leaf is not very well known and needs to be studied.

In the cobalt chloride method there is an additional source of error. During the time of observation carbon dioxide movement is hindered and the cobalt chloride paper prevents light from reaching the covered part of the leaf. As the time of such covering will vary greatly depending upon the rate of diffusion of water vapor from the leaf, another variable is introduced as a source of error.

The cobalt chloride method is probably the best among the indirect methods and has the advantage of quickness and practicality in field work over

any other method of stomatal study. It was not used in the present investigation, however, as the strip method, in many ways, seemed more reliable.

The three direct methods for studying stomatal behavior are (a) the LONG and CLEMENTS' collodion method, (b) the direct visual method, and (c) LLOYD's strip method.

In 1934, LONG and CLEMENTS (17) published their paper on the collodion method, discussing the method in detail. Apparently, the advantage of this method was that the same leaf could be used over and over again. But it was noticed early in my own trials that the leaves of *Tradescantia* often cooled more than 15° C., after the collodion was applied. It is doubtful whether this method does away with this disadvantage of LLOYD's strip method. When an attempt was made to reduce this cooling effect by removing the strip earlier, there was great distortion in the direction of the pull.

Upon exactly duplicating the technique of the above authors, it was found that the shrinkage caused by evaporation of the solvents was not 3 to 4 per cent., as LONG and CLEMENTS claim, but about 10 per cent. This error could be ascribed to the method of getting the shrinkage data. They coated an eyepiece micrometer with collodion. After the film was dry it was stripped off. The reading on the strip was compared to the actual reading on the micrometer. They found that the shrinkage was about 3 to 4 per cent., which was correct. The micrometer, however, is a stiff object as compared with the usual types of leaves. Moreover, the collodion adheres more firmly to dry glass than to a leaf. The leaves of *Tradescantia* sp. are somewhat fleshy, but not thick. When the collodion membrane on such a leaf dries, a marked tension develops, often causing the leaf to curl. Shrinkage obtained in strips of collodion taken from a leaf is not the same as that of collodion strips taken from a glass micrometer.

The collodion method was not used because of these errors and because of the difficulty of getting exact measurements of the opening. It has almost no advantage over LLOYD's strip method except in cases where the latter could not be used, *e.g.*, where the material is comparatively stout, as in pods of *Phaseolus*. Unless a non-shrinking, rapidly drying material is found to replace the ordinary collodion, this method must be relegated to a secondary place.

Direct observation of stomata *in situ* under a microscope is considered by most workers to be the most reliable. This method can be used to advantage in plants with comparatively thin leaves and large stomata. Moreover, many of the plants, such as *Tradescantia* sp., are very difficult to strip when turgid. This plant, with its large stomata and small leaves, lends itself nicely to this method of stomatal investigation.

LLOYD (15) used a microscope with a small flask containing water as a condenser and heat absorber. LOFTFIELD (16) arranged a microscope stand



for direct observation but unfortunately neglected to describe his technique. Various individuals have described techniques for direct observation of stomata, but, as a whole, they are more suitable for counting the stomata per unit area of leaf than for measuring their aperture.

For the present investigation an apparatus was set up to study the stomata *in situ*. The accompanying diagram (fig. 1) shows some of the features of the apparatus. There are two strong vertical rods (2) about 1.5 inches diameter on which are fixed the horizontal rods (1 & 3) about 0.75 inches in diameter. The horizontal rod (1) is so fixed that by loosening the screw (8) the microscope can be slid along the rod (1) and can be taken to other plants mounted in the same manner as the flask (4) along the rod (3). The glass-slide stage (6), with leaf in position (drawn diagrammatically), is shown in operating position on the microscope. In the right corner of the illustration the glass-slide stage is drawn showing details of construction,  $\alpha$  representing material made of slightly stiff celluloid. The mirror (9) was swung out of position in the daytime while it was used to reflect light from a source not shown in the illustration. A small wooden piece (7) is tied to each of the vertical rods, only one of which is shown at (2). To these two wooden rods (7) was tied a soft string which supported the plants. At (5) is seen the old style microscope stand from which the base is removed. It is held in position by ordinary laboratory clamps. Objective lenses used were 4-mm. and 8-mm. Zeiss achromatic objectives with a 15 $\times$  ocular. The green color of the leaves served more or less as a color filter.

The condenser was eliminated from the apparatus and natural light was used during the daylight hours. At night or on a dull day, light from a 60-watt frosted Mazda electric lamp was reflected from the microscope mirror through the leaf to the microscope objective. It has been found that light from a 500-watt bulb with a bright reflector five feet from the leaf and an 8-inch electric fan four feet from the microscope caused a slight opening of the stomata in nine minutes, while easily visible opening took place in thirteen minutes. It was concluded from such experiments and others that the comparatively weak light from a 60-watt lamp at a distance of 5 feet for a maximum period of two minutes did not change the aperture to a noticeable degree.

Ordinary stages on microscopes are not only too large but they exclude light from the leaf during the period of observation. Moreover, with *Tradescantia* a slight carelessness in handling causes breaking at the growing point, making the specimen useless for further observation. For these reasons, the ordinary microscope stage was replaced by a glass slide.

LOFTFIELD put the leaf between a glass slide and a coverslip. This he clamped very lightly. This method not only causes slight pressure but partially prevents efficacious gas exchange. This was thought undesirable.

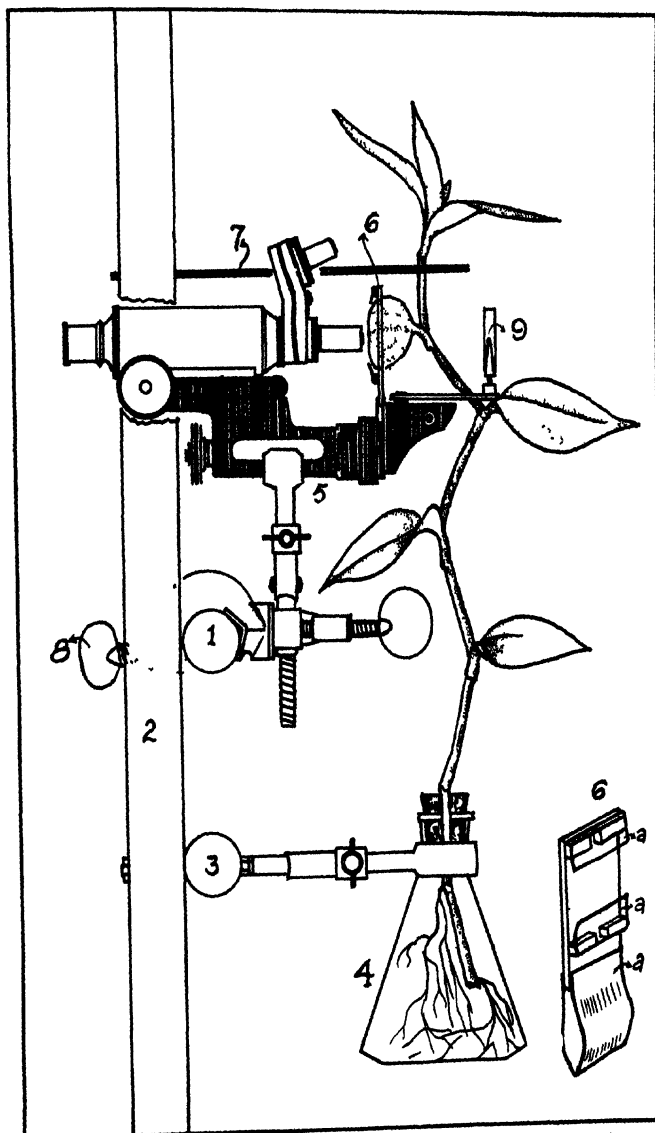


FIG. 1. Apparatus for direct visual observation of stomata *in situ*.

Two pieces of celluloid were mounted on a glass slide in such a way that a leaf could be slipped into position quickly and without undue disturbance. The illustration, figure 1(6), clearly shows this method. This arrange-

ment not only keeps the stomata from coming in contact with the glass slide (as this plant has no stomata on the upper side of its leaves), but also keeps the leaf flat so that all of the field is in focus.

With this apparatus the microscope can be slid from plant to plant without any trouble and a leaf can be brought under the field of the microscope with the least possible disturbance and change in the position of the leaf. The leaves of the plants were always left in their natural position and the microscope tilted as needed to get the leaf on the slide.

For observation with this method, two plants per treatment were selected and their leaves kept in as similar a position as possible with reference to light and convenience for microscopic observation. At the proper time the leaf was brought carefully into the field and 15 stomata rapidly measured.

This method is excellent when working with only one treatment. But when a comparative study has to be made of four treatments, as was the case in the present investigation, the method is rather a tax on the worker. LOFTFIELD did not measure more than ten stomata, but in the present work measuring 120 stomata per hour was very difficult. Because of this, no attempt was made to make very accurate measurements of stomatal apertures, as was done in the case of the strip method. The observations obtained by this method agreed essentially with those obtained by the strip method and hence the data reported are only for the strip method.

In the present case, direct observation *in situ* was tried not only with transmitted light but also with reflected light. The apparatus used was Leitz Ultropak. This microscope, though suited for stomatal counts, has not proved satisfactory for measurement of stomatal apertures. One of the reasons is that there is a large refraction from different parts of the walls of the guard cells making up the stomatal aperture. The situation was but slightly improved by the use of color filters, and other devices. Even if this difficulty was overcome, the microscope became useless in bright daylight in the greenhouse. Use of hoods over the head of the observer and ocular helped a little but the whole outfit became unwieldy in the small space of a greenhouse.

LOYD (14) first reported on his strip method in 1908. It was put on a firmer basis by LOFTFIELD (16). Since that time various individuals have utilized this method. The strip method has been found to be as exact as the direct ~~visual~~ method if the precautions mentioned by LOFTFIELD are strictly adhered to and if observations are made on material mounted in absolute alcohol instead of the original mounting in balsam. But the instruments employed by LOFTFIELD were too cumbersome to manipulate without much practice. It has been found that a sharp, pointed scalpel in conjunction with the thumb of the right hand is all that is necessary for speedy stripping. ERNEST (5) has used a similar technique in her study of suction-

tension gradients. With this simplification it should not take more than one second to get a strip and plunge it into a vial of absolute alcohol.

Many plants have leaves which cannot be stripped easily. Plants like *Zea mays* and *Phaseolus* do not yield good strips when deficient in phosphorus or nitrogen. Here the veins are a little closer together, causing the strips to break easily. Some plants, like *Pyrus malus* and others, have leaves distinctly divided into small islets by the close, coarse venation. In this case the leaves are often quite brittle when folded between the fingers. Such leaves seldom give good strips, while plants with more pliable leaves very often can be stripped easily.

Moreover, some leaves (*e.g.*, *Pisum* and *Impatiens* sp.) can be stripped much more easily when the strip is taken in a region where the distance between two major lateral veins is appreciable, and where minor anastomosing veins are not too prominent. In such a case, if the strip is begun nearer the midvein and carried out towards the margins, a long piece of epidermis can be obtained with ease. In the grasses and in all leaves with parallel venation, the strip could be profitably started between two large parallel veins and pulled out towards the tip. From *Zea mays* the writer has been able to obtain strips as long as 1 cm. and a little less than 5 mm. wide with a very small amount of tissue from the palisade region. A whole leaf of *Pisum* can be stripped in one piece with little difficulty.

While investigating these methods, a simple test of so-called "stripability" of leaves was accidentally hit upon. It was found that if a leaf from a plant gives an epidermal strip easily when peeled by means of the nails on the thumb and second finger, the plant may be taken as good material for stripping. This method should be tried when the leaves are turgid, since in many plants the leaves strip rather easily when slightly wilted but not when turgid (*e.g.*, *Tradescantia* sp.).

A troublesome feature of the strip method is the large number of vials needed when stomatal behavior in variously treated plants is being studied. To eliminate this difficulty, bags were made from "onion tissue paper." LePage's liquid glue was used because it is practically insoluble in absolute alcohol. Small paper bags (6.5 × 3.5 cm.) were prepared and kept in absolute alcohol. After about twenty-four hours this alcohol was replaced with fresh alcohol. The strips were plunged into vials (about  $\frac{1}{3}$  full) containing absolute alcohol, left there for about fifteen minutes, and occasionally shaken to prevent dilution of alcohol around the strip. The shaking of vials is quite important as a small amount of leaf tissue often accompanies the strip. After this the strips were transferred to the bags and left there till needed. Generally the bags were marked in pencil with information about the strip before they were transferred. The paper bags were stored in well-stoppered wide-mouthed bottles containing absolute alcohol.

Whenever required for making the measurements, the strips were removed from the paper bags, trimmed, mounted on a clean, dry glass slide and covered with a no. 1 square coverslip. *The mounting medium was absolute alcohol.* The coverslip was sealed to the glass slide by means of ordinary beeswax. In no stage of this processing were the strips allowed to become dry.

Early in the work it was noted that mounting the strips in Canada balsam (with xylol as solvent) brought many post-mortem changes in the stomatal aperture. This was noted very often in *Zea mays*. For this reason the usual technique was abandoned and the material was mounted in absolute alcohol. This not only does away with the most important defect of LLOYD's strip method but also saves an enormous amount of time in the processing. Observations regarding the post-mortem changes in stomatal postures have been amply corroborated by the work of NADEL (25). The writer agrees with OPPENHEIMER (26) in that much could be gained from the study of individual stomata, but he does not think that conclusions based on the study of a population of stomata are very far removed from those arrived at by the study of an individual stoma. The criticism of OPPENHEIMER (26) of the earlier work on stomata using the strip method is valid, but is not pertinent to the work reported here.

In this work it was noted that staining is not necessary with the ordinary type of stomata, but it is with stomata of the grass type. In order to obtain uniform results with these, with a minimum expenditure of time, all the bags containing the strips were placed in the staining jar at the same time. After the right stage of staining was attained, the bags were cleared twice in absolute alcohol. The strips were then removed, trimmed, and mounted as usual.

In the case of stomata of the ordinary type, at least thirty were measured under a 1.8-mm. oil immersion objective and a 10× Bausch and Lomb filar micrometer. These were taken from 2 or 3 strips obtained from at least two plants. This insures much more accurate measurements of stomatal apertures than could be obtained with ordinary ocular micrometers. In the case of the grass type, a measurement of about 60 to 100 stomata per treatment per reading was the rule. This largely eliminated the error of measuring a small number of stomata. Thus the objection raised against the strip method by ASHBY (1) and others is not pertinent here. Even by measuring fewer stomata, ASHBY has found that the stomatal curves, obtained by the strip method and the porometer method, run close together, except where the stomata are nearly closed. The error here probably lies with the porometer method.

KNIGHT (13) points out that the weakness of the strip method is the violent treatment accorded to the leaf. Some think that holding a leaf be-

tween two fingers momentarily (as is done while stripping) is a violent treatment, but the observations of SCARTH, WHYTE, and BROWN (32) do not corroborate it. SCARTH (31) observed that "mechanical stimulation of the plant or leaves by shaking, rubbing, exposing to blasts of air, etc., which was found by KNIGHT to induce partial and temporary closure in certain plants, seems to have no effect on *Zebrina pendula* or (so far as observed) on any species" with which he worked. My experience agrees essentially with that of SCARTH. If cutting a leaf, as is done in stripping, is a violent treatment then this difficulty can be overcome by not using the same leaf twice. This was the practice in the present investigation, although the observations of SCARTH *et al.* (32) indicate that the injurious effect does not extend beyond a millimeter or so from the actual wound. When he allowed the "shaved off" epidermis to be immersed in water, visible closure began within a few minutes and became practically complete in about fifteen minutes. The stripping in the present method is so rapid (not taking more than a second or so) that there is very little chance for the stomatal aperture to change enough to be measured by a Filar micrometer. "Stripping" of the epidermis, as is done in the present work, has been found to be better and speedier than the "shaving" or the "slicing" technique of various workers. This is especially true when stomatal apertures are studied in fixed preparations.

As a quantitative method, the strip method, if carefully used (with the modifications reported here), seems to be the best for the study of stomatal behavior. It is superior to the direct visual method in that there is less strain on the worker's eyes. It gives as accurate data as the direct visual method and, for a longer period of observation, it is probably even more accurate because there is no fatigue. It allows rechecking of observations. Another important advantage of the strip method is that stomatal behavior observed during a period of 12 or 24 hours is not that of one or two plants only but of a population of plants; hence it reveals more accurately the probable behavior in plants than the porometer or the direct visual methods. But the advantage of the porometer method is evident when the gaseous exchange through one particular set of stomata is to be measured. All the methods except the porometer and the direct visual method entail the growing of a large number of plants.

#### CULTURAL METHODS

The cultural methods used during the course of the present investigation were water, sand, and soil cultures. The minimum number of replications per treatment was 20, the maximum, 50. In water and sand cultures a 1 to 10 dilution of modified Knop's solution was used, while in soil cultures the following solution was applied to each pot containing an average of 900 gm. of sandy loam soil:

1 gm. ....	$\text{KH}_2\text{PO}_4$
1 " ....	$\text{NH}_4\text{NO}_3$
20 mg. ....	ferric tartrate
1 liter .....	distilled water

This was applied in small amounts at intervals during the entire growing season of a series. Whenever a certain nutrient element was omitted from the standard culture solution it was replaced by equivalent amounts of some other commonly used element, such as sodium for potassium.

The present investigation was carried out during 1933, 1934, and part of 1935. Most of the experiments were carried out in the greenhouses at Cornell University. In each series the data were collected under a standard condition of air movement, *i.e.*, three 8-inch fans were kept continuously running under the greenhouse bench on the days when the data were obtained.

The purpose of the present work was to study the effect of deficiency and not the effect of total lack of these nutrients. No attempt was made to use refined cultural methods such as purifying the salts, etc. It was most important to have the plant make fair growth in spite of the deficiency. For this reason soil seemed the best medium in which to grow the plants. Here the plants generally showed clear-cut deficiency symptoms which could be checked by the recovery that took place when the deficient nutrient was added. In obtaining each series of data it was customary to see whether the symptoms noted at the time of taking the readings were due to deficiency of the nutrient not provided, or to deficiency of another nutrient. Some of the data where such complications prevailed were discarded.

## Experimental results

### A. DEFICIENCY SYMPTOMS

According to the experience of McMURTREY (24), KARRAKER and BORTNER (12), REED and HAAS (29), JACOB (11), and others, different nutrient deficiencies cause definite symptoms. These could be used more conveniently as criteria for nutrient deficiencies than cumbersome and uncertain analyses especially in controlled experiments. The deficiency symptoms observed here are, in the main, similar to those noted by other investigators.

In the full-nutrient cultures the plants were healthy, having a uniform green color in all of the leaves (figs. 2, 3). Here the older leaves continued to remain green for a longer period of time than in any deficient cultures. The dry weights were also generally higher, with the top-root ratio rather large. The roots were not longer than in the deficient cultures but were abundantly branched.

The plants grown in culture media deficient in potassium were generally smaller in size (fig. 10) than those grown in full nutrient cultures. The



FIG. 2. Symptoms of nutrient deficiency in leaves of *Nicotiana* (series TO2). Leaves were obtained from the basal region of plants shown in figure 10.

1. Supplied with full-nutrient solution
2. Deficient in potassium
3. Deficient in phosphorus
4. Deficient in nitrogen

margins and tips of the older leaves at first turned yellow and then showed brownish necrotic areas in the mesophyll tissue between the veins, but that close to the veins, as a rule, remained dark green (figs. 2, 3). Finally, the older leaves dried up, beginning with the tip and margins, and often remained hanging on the stem in a shriveled condition. As compared with the older leaves, the younger ones remained dark green with a bluish cast. The younger leaves did not show yellowing nor necrotic areas.

In contradiction to JACOB (11), no "falling over due to withering of the stem at the base" was noted in plants deficient in potassium. Also no accumulation of purplish or reddish pigment in veins was observed, although such pigmentation was noted on the margins and midrib in *Zea*, *Raphanus*, and other plants when deficient in nitrogen.

*Pisum* did not show any definite symptoms of potassium deficiency, except that they were slightly smaller and their older leaves dried earlier. Symptoms of potassium deficiency of the ordinary type have been noted in sweet peas (*Lathyrus odorata*). The top-root ratio in plants suffering from potassium deficiency was not as large as in those grown in full nutrient cultures (table III).

When not supplied with phosphorus, the leaves of the plants developed a dark green color with a dusky or brownish tinge. The leaf size was greatly reduced (fig. 2). *Nicotiana* plants have been observed to show development of ochre brown spots (fig. 3), especially in the older leaves. This condition has appeared consistently in all of the experiments with *Nicotiana* and does not seem to be due to chance. A similar type of spotting was noted by KARRAKER and BORTNER (12), and McMURTREY (24), but the latter author





FIG. 3. Symptoms of nutrient deficiency in leaves of *Phaseolus* (series B1) Leaves were obtained from the basal region of the plants, where deficiency symptoms are markedly shown. Stomatal behavior in this series is shown in figure 6.

1. Supplied with full nutrient solution
2. Deficient in potassium
3. Deficient in phosphorus
4. Deficient in nitrogen

is not sure that this spotting was due to phosphorus deficiency. In the present work on *Nicotiana*, the severity of this spotting decreased when the plants were supplied with phosphorus. No spotting of this type has been observed in *Phaseolus* or other plants used. This contradicts the observation of JACOB (11), who noted spotting in *Phaseolus* deficient in phosphorus. However, this may be a matter of relative deficiency.

In the case of *Tradescantia*, the leaves became mostly small, with the similar brownish deep green color and a deep purplish tinge over the lower

side. The leaves became fleshy and very brittle when folded, which is in agreement with the observation of PRIDHAM (28). In all cases, the phosphorus deficiency caused rapid drying of the lower leaves. The dried leaves generally showed a dark brown color. *Pisum* again showed no definite symptoms, corroborating JACOB (11). The internodes were longer in *Tradescantia*, *Phaseolus*, and *Zea*, and the plants were spindling. In practically all cases recovery took place when the plants were supplied with phosphorus in the form of phosphate.

In the plants deficient in nitrogen, there was a definite restriction of new growth, as in the phosphorus-deficient plants. The internodes, on the contrary, were short, giving the plants a rosette-like appearance. The leaves were smaller, thicker, and more brittle in *Tradescantia*, and showed purplish margins. A slight pink coloration was also noted on the lower side of the leaves of this species.

The top-root ratio was rather small in plants deficient in nitrogen, which is in agreement with the findings of various workers. In this regard it could be stated that the top-root ratio can be changed more or less markedly depending upon the severity of the deficiency. In one case with *Zea*, the plants deficient in nitrogen had a higher top-root ratio than those deficient in phosphorus (table III), while in another case the reverse was true. In these cases the stomata were more responsive in the plants with a larger top-root ratio.

In the cultures deficient in nitrogen or phosphorus, the veins are closer than in plants grown in full nutrient solution or one lacking in potassium. This results in considerable difficulty in obtaining large strips from plants deficient in nitrogen or phosphorus.

The deficiency symptoms are clear-cut in all of the plants used except in *Pisum*. Most plants show the effects of deficiency very markedly, and it is easy to pick out a plant lacking a certain nutrient. In general, recovery takes place when the deficient element is added, except in the older leaves or where the deficiency is very severe and far advanced.

#### B. STOMATAL BEHAVIOR

In the preliminary experiments, the soil was not mixed with river sand (to make it a poor sandy loam soil), and, as a result, no definite deficiency symptoms were observed. Here the plants were of a healthy green color in all the treatments, but those with full nutrient and those deficient in potassium were often slightly taller. The stomatal behavior in these plants did not show any definite differences. The differences obtained were so small as to be within the range of experimental error. Figure 4 shows the result of one of the experiments of this class with *Zea mays*.

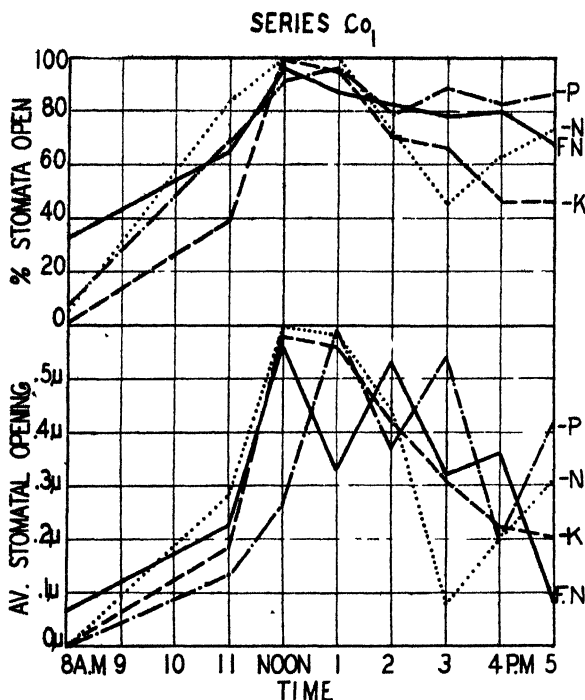


FIG. 4. Stomatal behavior in leaves of *Zea mays*.

The conclusion that stomatal behavior is not markedly affected by nutrient deficiency unless the plants show some definite signs of deficiency is essentially correct, as shown by an experiment with *Pisum* (fig. 5). In this experiment, the roots grew out of the flower pots and obtained nutrients from the cinders with which the greenhouse bench was covered at that time. The cinders had enough potassium for normal growth. The stomatal behavior was practically normal in this case.

The cultures with no nitrogen added (fig. 5), even though well sterilized at the start, became infected by legume bacteria so that abundant root nodules were formed. (The infection, as shown by the size and number of nodules, was greater in plants not supplied with nitrogen as nutrient than in the plants supplied with nitrogen.) The growth in the plants supplied with nitrogen by bacteria only was more like that of normal plants than is the case with plants really deficient in nitrogen. In this case, the stomatal behavior showed a trend toward that of the healthy plants. Although the nitrogen was supplied to these cultures through the nodule organism, it was not enough to make the plants grow as vigorously as when the full-nutrient solution was applied. The stomatal responses were not so quick as in the plants with the full nutrient or those deficient in potassium. This was especially true in the early daylight hours.

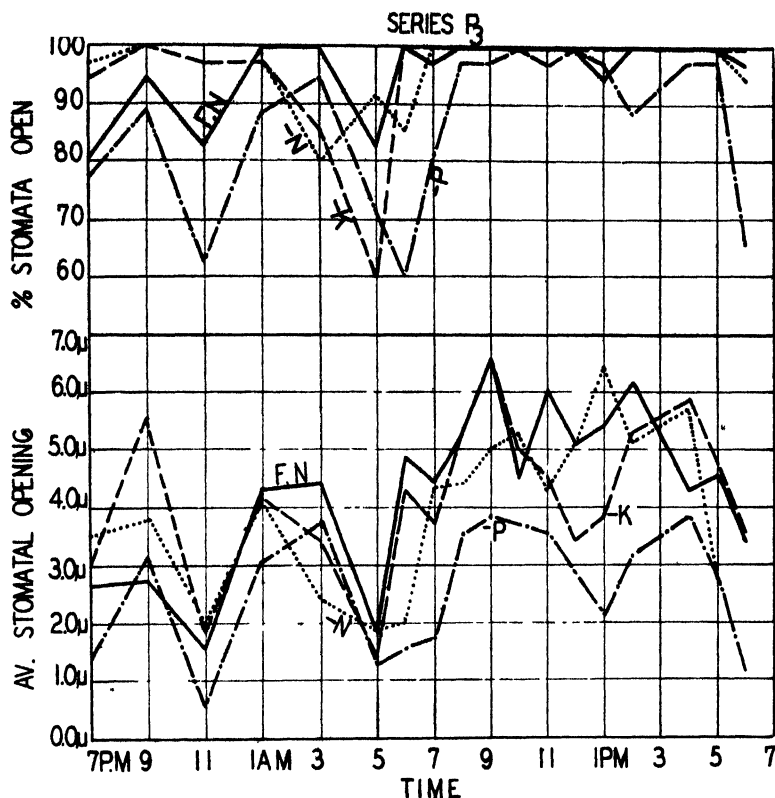


FIG. 5. Stomatal behavior in leaves of *Pisum*.

The cinders were not rich in available phosphorus and the stomatal responses were sluggish, especially in light, in plants where this element was not supplied. In this case, even though the plants showed no specific deficiency symptoms, they were decidedly affected in their growth.

In other sets of experiments no cinders were left on the bench, and most of the series, except *Pisum*, showed clear-cut deficiency symptoms. Whenever these symptoms were obtained, stomata were found to be decidedly subnormal in their responses to the environmental conditions, especially to light. The data of this type, from two of the experiments with *Phaseolus*, are given in figures 6 and 7.

In the first experiment (fig. 6), when all the nutrients were supplied, the stomata showed quick response to changes in light in the morning. Rapid closure of stomata took place with the first signs of wilting (about noon). As the plants recovered from wilting, opening took place. The plants deficient in potassium showed the same general behavior but it was less marked than in the case of the full-nutrient cultures. The leaves of plants with no

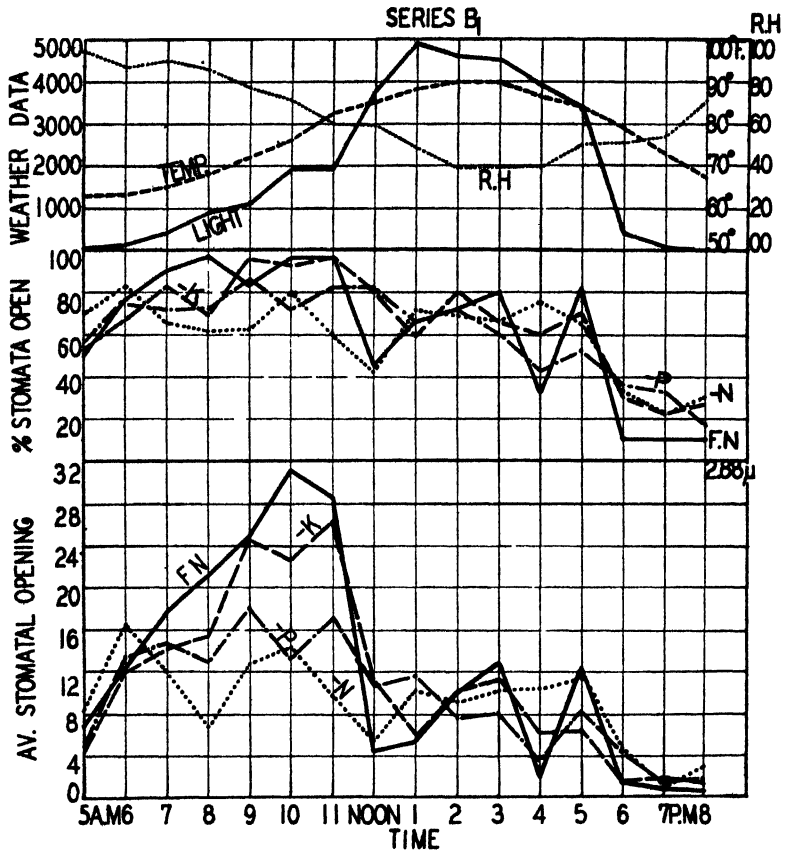


FIG. 6. Stomatal behavior in leaves of *Phaseolus*.

phosphorus or nitrogen were flaccid to the touch at about noon but showed no visible signs of wilting. Here the stomatal responses were slight compared to the other two. In the case of these two cultures, there seems to be no relation between light and stomatal behavior. Some other factor seems to be controlling the stomatal responses.

In the second experiment (fig. 7), the plants were not watered at the beginning as in the previous one. In the plants with full-nutrient supply stomata responded quickly to the changing light conditions. With the onset of wilting, the stomata closed rapidly. At this time (8 A.M.), all the plants were watered, hence they recovered from wilting and the stomata opened.

The plants deficient in potassium showed the same general stomatal behavior but the variations were less marked. The plants deficient in phosphorus or nitrogen showed narrower opening, as usual, though the trend in general response was similar to other cultures.

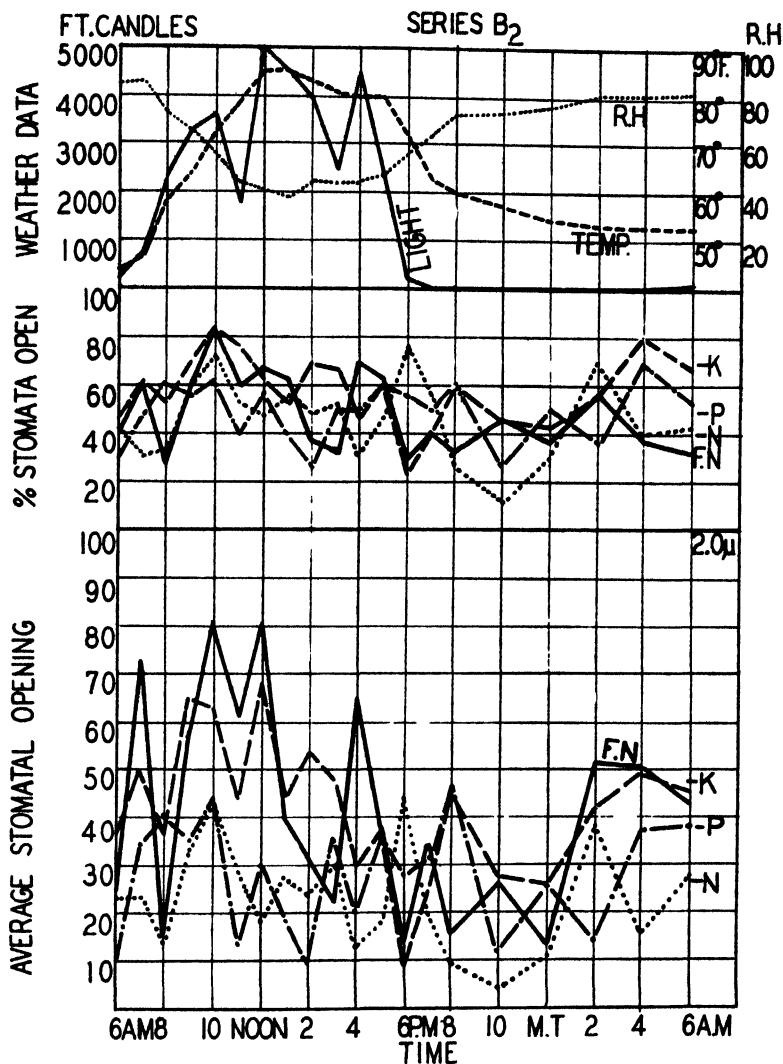


FIG. 7. Stomatal behavior in leaves of *Phaseolus*.

About 11 A.M. (fig. 7), the sky became cloudy. All cultures showed similar stomatal responses. The light was bright again at 12 M. and all cultures except those deficient in nitrogen showed stomatal opening. In this last case the opening response was quite sluggish. At 1 P.M., the plants in full nutrient cultures showed signs of wilting while those deficient in potassium or phosphorus appeared flaccid to the touch but did not show visible signs of wilting. The stomatal activities were what would be expected from previous experience.

The data from these two experiments with *Phaseolus* represent typical data obtained in other experiments with *Tradescantia* and *Pisum* in water cultures, as well as in experiments in soil cultures with *Nicotiana*, *Zea*, and several other plants.

### C. NIGHT OPENING OF STOMATA

Throughout the course of the present work (figs. 5 and 7), night opening of the stomata has been noted consistently in many plants, notably in *Phaseolus* and *Pisum*. The writer has been unable to find night opening in *Zea*, which agrees with the results of LOFTFIELD (16). This phenomenon is not due to any experimental error as some claim, but is real, because the writer has not only seen night opening of stomata that were removed by the strip method but also of stomata observed by the direct visual method. SCARTH *et al.* (32) have confirmed the truth of this statement.

The *Pisum* plants deficient in potassium have often shown a larger percentage of stomata with wider apertures at night than those plants grown in other cultures (fig. 5). If the CO<sub>2</sub> content of the guard cell vacuome affects the stomatal behavior, then the explanation of GREGORY and RICHARDS (8) may be true in this case, for they found a higher respiratory rate in plants with deficient potassium. Unfortunately, the technique of these investigators is not above reproach, so nothing definite can be said.

### D. EFFECT OF EXCESS POTASSIUM ON STOMATAL BEHAVIOR

In one of the experiments with *Nicotiana* (fig. 8), the pots were half buried in cinders at the beginning. As the pots were a little larger (6-inch) than the standard 4-inch pots, the nutrient supply was doubled. The plants not supplied with potassium obtained an adequate amount of it from the cinders and made the best growth, while the plants supplied with all of the nutrients showed a mottling of their lower leaves, which was followed a little later by shriveling and drying. HILL, DAVIS and JOHNSON (10) have noted in chrysanthemums a similar injury due to excess of potassium. Apparently, then, excessive and deficient amounts of potassium produce somewhat similar symptoms.

In this case, the total stomatal opening was practically the same in cultures not supplied with potassium as in those cultures receiving the full nutrient supply, but the stomata were more responsive in the former. This may explain the findings of GASSNER and GOEZE (7) who, when they used three different amounts of potassium supply, found that the highest carbon dioxide assimilation occurred with one-twentieth of the normal supply. With increasing concentration, they obtained a decrease in assimilation. They also found a decrease in assimilation with a deficiency of potassium.

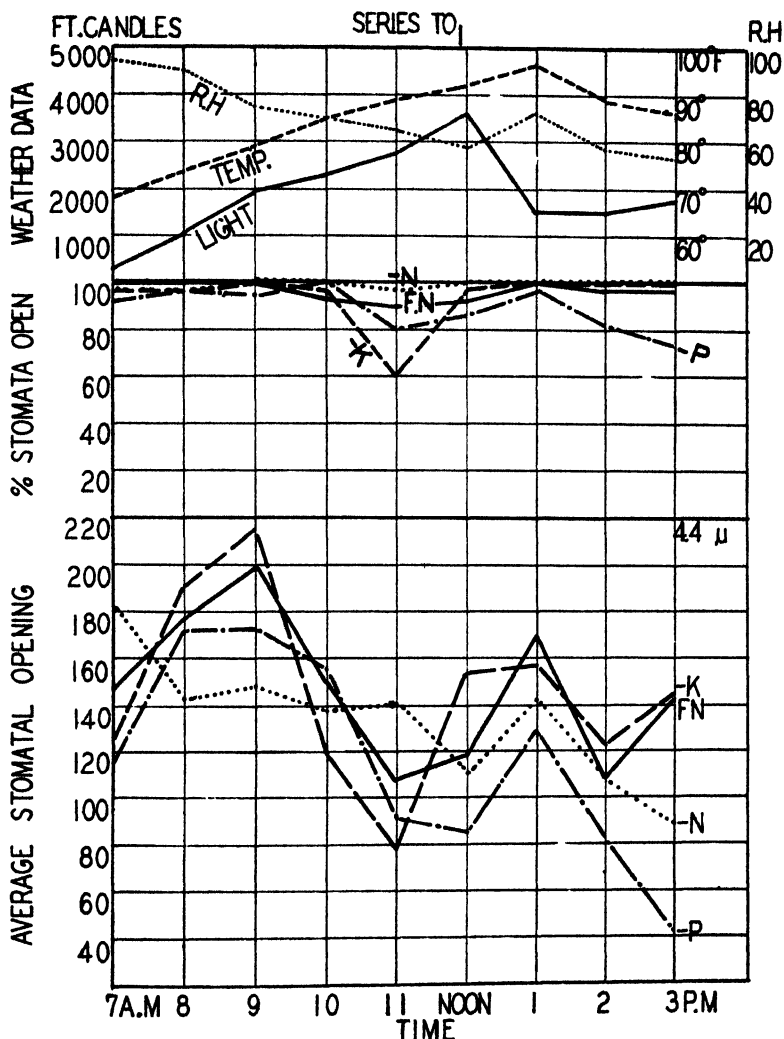


FIG. 8. Stomatal behavior in leaves of *Nicotiana*.

In this series, cultures deficient in phosphorus or nitrogen showed less wide stomatal opening than was found in the cultures discussed above. The total opening was low in cultures deficient in phosphorus as compared with those deficient in nitrogen. This again showed a correlation between the size of the plants and their stomatal behavior.

#### E. EFFECT OF NUTRIENT DEFICIENCY ON STOMATAL SIZE

Casual observations showed no appreciable differences in the sizes of stomata from variously treated plants. In one of the experiments with



*Nicotiana*, very careful measurements were made. In this case not only the size of the stomata was noted but also the width of the aperture. The results were surprisingly different from what one would expect. The stomata were slightly larger in the plants supplied with deficient nutrient solutions than in the normal ones (tables I, II).

TABLE I  
SIZE OF STOMATA IN *NICOTIANA* (SERIES TO2)

TREATMENT	SIZE OF APERTURE*	NO. OF STOMATA MEASURED†	SIZE* OF STOMATA IN MICRONS	
			AV. WIDTH	AV. LENGTH
	μ		μ	μ
Full nutrient .....	0-0.2	4	30.0	30.5
Potassium-deficient .....	"	12	34.3	31.7
Phosphorus-deficient ...	"	29	35.7	32.0
Nitrogen-deficient .....	"	1	34.0	26.0
Full nutrient .....	1-1.2	4	34.1	31.7
Potassium-deficient .....	"	4	31.7	34.8
Phosphorus-deficient ...	"	26	37.3	33.5
Nitrogen-deficient .....	"	10	38.6	35.0
Full nutrient .....	2-2.2	53	34.8	33.2
Potassium-deficient .....	"	41	34.6	34.2
Phosphorus-deficient ...	"	44	37.5	34.2
Nitrogen-deficient .....	"	50	36.4	35.9
Full nutrient .....	3-3.2	21	35.5	33.9
Potassium-deficient .....	"	23	35.4	34.8
Phosphorus-deficient ...	"	9	38.4	35.3
Nitrogen-deficient .....	"	14	37.1	35.7
Full nutrient .....	4-4.2	42	36.0	34.8
Potassium-deficient ...	"	38	36.1	35.2
Phosphorus-deficient ...	"	25	37.4	35.7
Nitrogen-deficient .....	"	25	37.8	36.6

\* Measurements of stomatal aperture and stomatal size in tables I and II were obtained with a filar micrometer (Bausch and Lomb 10x ocular) and a 1.8-mm. oil immersion objective.

† These data were obtained from strips obtained in series TO2 during a period of 13 hours (7:00 A.M. to 7:00 P.M. inclusive).

These data apparently contradict the findings of LUTMAN (19), who observed that with nutrient deficiency the stomatal size decreased in many cases. It seems that the technique used in obtaining the data may contribute to this contradiction. It has been emphasized before that, although the plants showed deficiency symptoms, they did not die but often flowered (fig. 10),

TABLE II  
AVERAGE STOMATAL SIZE\* IN *NICOTIANA* (SERIES TO2)

TREATMENT	NO. OF STOMATA MEASURED	SIZE OF STOMATA	
		AV. LENGTH	AV. WIDTH
		$\mu$	$\mu$
Full nutrient	124	34.1	32.8
Potassium-deficient	118	35.6	34.1
Phosphorus-deficient	133	37.3	34.2
Nitrogen-deficient	100	40.8	33.8

\* Stomatal measurements for each treatment in table I were averaged without regard to the size of the stomatal aperture and are reported in this table (II).

and fruited to a certain extent, *e.g.*, in *Pisum* and *Phaseolus* (table VI). Also, the stomatal measurements were obtained from the third or fourth leaf from the tip of the stem. LUTMAN's data were obtained from comparatively immature plants so that his choice of leaves was limited, with the result that he could not use a definite leaf or portion of a leaf, as was done in the writer's experiments. Owing to this and other reasons, the data are not comparable and the whole problem remains unsettled.<sup>1</sup>

#### F. EFFECT OF NUTRIENT DEFICIENCY ON STOMATAL DISTRIBUTION

It has been the experience of the writer that the plants treated with all of the nutrients showed a much more uniform distribution of their stomata than the deficient plants. This was especially true of the plants deficient in nitrogen, in which the stomata showed a tendency towards arrangement in groups instead of being evenly distributed. In other deficient cultures this was true, but to a lesser degree.

In figure 9 are given data on the distribution of stomata in one of the interesting cases discussed in section D. Here the full nutrient plants had an excess of potassium, while the plants supposedly deficient in this element got enough of it from the cinders. Stomatal behavior showed the effect of excess potassium (fig. 8). It will be seen that the plants receiving adequate potassium show uniform distribution of their stomata, while the plants with excess potassium do not show as uniform stomatal distribution. But the cultures deficient in phosphorus or nitrogen show a very uneven distribution of stomata as compared with the first two.

In these experiments it has been found that the average number of stomata per unit area of the leaf is not very much affected. The variations are not

<sup>1</sup> The problem of size of stomata and other cells, in these variously treated plants, is being studied by Dr. ERNST ABBE and hence the writer has not developed the subject further.

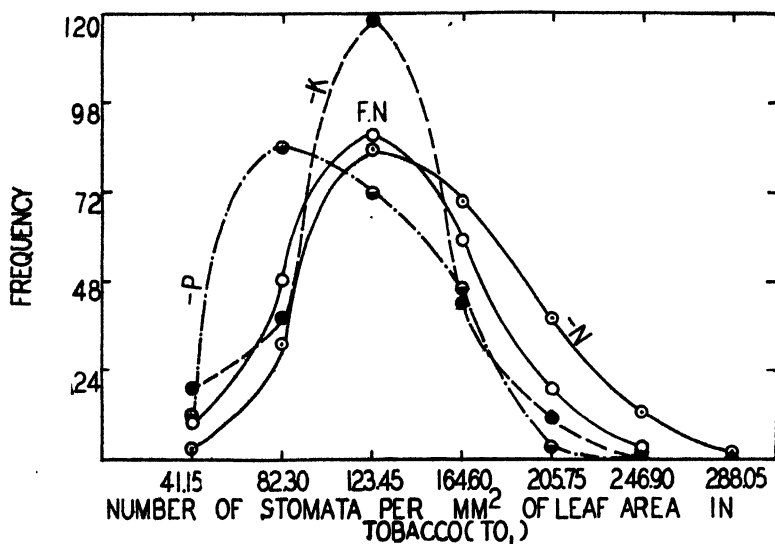


FIG. 9. Distribution of stomata in leaves of *Nicotiana*.

large and show no definite tendency towards decrease or increase with a deficiency of nutrients. The observations of PLEASANTS (27) are verified in this regard. The data presented in table III are from the experiment discussed in the above paragraph.

TABLE III  
NUMBER OF STOMATA IN *NICOTIANA* (SERIES TO1)

TREATMENT	AV. NO. OF STOMATA PER MM. <sup>2</sup> OF LEAF AREA
Full nutrient (actually had excess of potassium) .....	130.5
Potassium-deficient (obtained potassium from cinders)...	121.0
Phosphorus-deficient .....	114.8
Nitrogen-deficient .....	149.0

#### G. CORRELATION BETWEEN STOMATAL BEHAVIOR AND OTHER PERFORMANCES OF THE PLANT

In the plants studied it was noted repeatedly that there was a definite relationship between the size of the plant, the yield of the fruit, the water requirement, and the stomatal behavior (tables IV, V, VI, and fig. 10). The plants grown with all of the nutrients were generally larger, had heavier yields (table VI), and a lower water requirement (table IV). The stomata also showed active responses to changes in environmental conditions (figs. 6, 7).



FIG. 10. Size and appearance of *Nicotiana* plants (series TO2) when grown in culture media lacking various nutrients.

1. Plants supplied with full-nutrient solution
2. Plants deficient in potassium
3. Plants deficient in phosphorus
4. Plants deficient in nitrogen

TABLE IV  
WATER REQUIREMENT IN *ZEA* (SERIES C2)

TREATMENT	FRESH WEIGHT		TOP-ROOT RATIO	AMOUNT OF WATER LOST IN THREE WEEKS	WATER REQUIREMENT PER UNIT FRESH WT.	
	TOP	ROOT			TOP	ROOT
	<i>gm.</i>	<i>gm.</i>		<i>gm.</i>		
Full nutrient	81.60	24.38	3.34	377.33	4.624	15.47
Potassium-deficient	68.33	36.13	1.88	413.00	6.044	11.43
Phosphorus-deficient	11.82	9.60	1.22	145.66	12.323	15.17
Nitrogen-deficient	7.27	6.43	1.13	181.00	24.896	28.15

When the plants are a little smaller, as in the cultures deficient in potassium, the yield is less, and there is an increase in water requirement. The stomatal response is not so great as in plants supplied with all of the nutrients (table V).

With plants deficient in phosphorus or nitrogen, the order of stomatal aperture and response, water requirement, and yield varies, depending upon the size of the plants. If the plants deficient in phosphorus are smaller, then their water requirement is larger, their yield is less, and their stomatal response is sluggish. If they are larger than the plants deficient in nitrogen, these data are reversed.

**TABLE V**  
AVERAGE STOMATAL OPENING IN *PHASEOLUS* (SERIES B1)

TREATMENT	AVERAGE STOMATAL OPENING PER HOUR IN MICRONS*
	$\mu/hr.$
Full nutrient .....	1.08
Potassium-deficient .....	0.99
Phosphorus-deficient .....	0.86
Nitrogen-deficient .....	0.82

\* Measurements were made with a filar micrometer; observations were from 5 A.M. to 8 P.M. inclusive.

**TABLE VI**  
YIELD IN *PHASEOLUS* (SERIES B3)

TREATMENT	TOTAL YIELD	NO. OF PLANTS USED	AV. YIELD PER PLANT
	<i>gm.</i>		<i>gm.</i>
Full nutrient .....	584.1	24	24.337
Potassium-deficient .....	489.6	25	19.584
Phosphorus-deficient .....	123.3	25	4.932
Nitrogen-deficient .....	110.0	25	4.400

It seems, then, that there is a clear relationship between stomatal behavior and the general metabolism of the plant. The stomatal behavior is conditioned by the vigor of the entire plant, and is not independent of it. The same conclusion is arrived at when one considers the effect of water relations of a plant on its stomatal behavior.

### Discussion

From the data obtained during the course of the present investigation, it seems fairly clear that whenever the plants are affected by nutrient deficiency so that definite deficiency symptoms appear the stomatal behavior is changed. This change, in general, tends towards a less active response by the stomata to light, water, and other environmental factors. This low response is produced not only by a deficiency of these nutrients but also by an excess of potassium. This is especially true when this excess supply of nutrient causes injury of some sort. Such low response appears to be connected with the general metabolism of the whole plant. (It is reasonable to conclude from this that any factor that may affect the general metabolism of a plant may affect the stomatal behavior.)

From the work of SCARTH (31) and others it is fairly apparent that the pH of the guard-cell vacuome in some way conditions stomatal behavior.

Explanations for the possible effect of nutrient *deficiency* on stomatal behavior will revolve around this fact.

At first glance, it seems as if nutrient deficiency may change stomatal mechanism itself, *i.e.*, change the kind and amount of proteins in the guard cells. This would be true in the case of plants grown in culture media in which the plant growth is almost completely restricted, as in the experiments of LUTMAN (19). But such a viewpoint does not seem probable for the experiments reported here, as the stomata of plants from all the cultures have a capacity to show active response, as seen from the wide opening when placed in dilute ammonium hydroxide solution. Moreover, the stomata are sometimes a little larger in these deficient plants (*cf.* section E). Furthermore, the responses do not seem to be related very closely to any visible changes in amount of carbohydrates or of protoplasm.

Another explanation suggested by PLEASANTS (27) and supported by the work of SCARTH (31) and his collaborators (32) is interesting and more probable. This explanation is based chiefly on the fact that there exists a correlation between stomatal responses and fluctuations in the pH of the guard-cell vacuome. If the photosynthesis is higher in full-nutrient plants, the shifts in pH should be greater and, as a result, stomatal responses should be more pronounced. If the nutrient deficiency in some way reduced this photosynthetic activity, the shifts in pH should be small, giving lethargic responses by the stomata.

Unfortunately, studies of the effect of nutrient deficiency on plant processes like photosynthesis and respiration show no general agreement. To LUNDEGÅRDH (18) this difference in results seems to be due to the use of different plants and of leaves of different ages. The writer's own experience has shown that use of leaves of different location and different age can partly account for these contradictions. Yet, the effect of nitrogen deficiency on photosynthesis seems to be proved to a reasonable degree. It has been shown that nitrogen deficiency decreases the amount of chlorophyll. According to FLEISCHER (6), there is a decrease in the rate of photosynthesis with the decrease in chlorophyll content in the alga *Chlorella*.

The lower water requirement of the plants grown in well-fertilized soils is due to the fact that during the period when water loss is rapid, photosynthesis is very high. A slight decrease in the water content of the leaf most probably will not hinder greatly the normal rate of photosynthesis. This has been shown to be true in the case of *Pyrus malus* by CHILDERS (unpublished data). The water loss from stomata becomes less due to a reduced diffusion gradient. When wilting takes place, stomata close rapidly and thereby almost completely stop stomatal transpiration. But the translocation of the accumulated photosynthate will continue. The leaf is then ready to start photosynthesis at a very high rate when the leaf recovers from wilting and the stomata open.

In the case of cultures deficient in nutrients, on the other hand, there are smaller leaf surfaces, fewer leaves per plant, and a relatively larger root system as compared to the amount of top growth. Here the diffusion gradient remains steep during the course of the day resulting in excessive water loss. The amount of photosynthate manufactured during this time will be comparatively less for the amount of water transpired, *i.e.*, the amount of water transpired per unit of dry weight produced (or the so-called water requirement) will be large.

### Summary

1. In the present investigation the possible effects of nutrient deficiency on stomatal behavior were studied in *Zea*, *Pisum*, *Phaseolus*, *Nicotiana*, *Tradescantia*, and other plants. The nutrients studied were potassium, phosphorus, and nitrogen.

2. Stomata observed were those in the distal third of the lower surface of the third or fourth leaf from the growing stem apex.

3. All the methods available for the study of stomata were carefully tested. The direct visual method and LLOYD's strip method were found to be the best. Of these two, the strip method has many advantages over the direct visual method. Certain sources of error in LLOYD's original strip method have been done away with and other improvements in this method are suggested.

4. On the whole, the general deficiency symptoms are similar to those described by other workers.

5. Whenever the plants showed striking deficiency symptoms, stomatal behavior was decidedly subnormal. In the full-nutrient cultures, the plants showed their stomata to be more responsive to changing environmental conditions than when deficient in either potassium, phosphorus, or nitrogen.

6. Night opening of stomata has been observed in practically all the plants used except *Zea*.

7. With excess potassium, the plants were slightly smaller, their lower leaves mottled, and their stomata showed slight lethargic movement.

8. The number of stomata per unit area does not vary greatly, but their distribution is much more irregular in deficient plants. In one case, a tendency towards increased stomatal size in deficient cultures was noted, but in other cases there was no noticeable variation in size.

9. It seems that stomatal behavior reflects the general metabolic condition of the plant. The subnormal stomatal behavior is accompanied by increased water requirement, decreased yield, and decrease in the size of the plants. The average stomatal aperture is proportional to the size of the plant as determined by nutrition, *i.e.*, the widest opening occurs in the largest plant.

10. The viewpoint that leaf activities, *i.e.*, photosynthesis, respiration, and transpiration, control the stomatal behavior appears to be correct. That the condition of stomatal mechanism itself does not change in deficient cultures is indicated by the fact that when placed in ammonium hydroxide solution the stomata in all cultures respond similarly.

The writer wishes to thank Dr. O. F. CURTIS, for suggesting the problem and for advice and direction of the work. Thanks are also due to Dr. LEWIS KNUDSON, and Dr. L. W. SHARP for their assistance and criticism of the manuscript.

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# THE CONTINUOUS MEASUREMENT OF PHOTOSYNTHESIS, RESPIRATION, AND TRANSPIRATION OF ALFALFA AND WHEAT GROWING UNDER FIELD CONDITIONS<sup>1</sup>

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(WITH TEN FIGURES)

## Introduction

The apparatus and experimental technique which are described in this paper have been developed to determine *continuously, automatically, and throughout the life of a plant*, if desired, the carbon dioxide exchange and the transpiration in a chamber in which plants are growing under field conditions. This discussion gives a general résumé of our methods, including, first, a description of the equipment; second, illustrations of its application to alfalfa and wheat; third, a statistical analysis of the data, showing the magnitude of the errors and variability involved; and fourth, some interesting observations on the relation of light intensity and temperature to the rate of photosynthesis, respiration, and transpiration, which these methods have made clear.

The lack of an adequate method for the rapid and continuous determination of atmospheric carbon dioxide has been responsible for the somewhat limited employment of the carbon dioxide exchange in the measurement of photosynthesis of plants and respiration of both plants and animals. The best methods available—including the gasometric procedure of BENEDICT (2); the volumetric method of JOHNSON and WALKER (5); the electrometric method of SPOEHR and MCGEE (8); and the gravimetric methods—have left much to be desired either of convenience or accuracy for this purpose. Since the atmosphere contains only 310 parts per million by volume of carbon dioxide, the gasometric and gravimetric methods have been handicapped by insufficient material, and the volumetric and electrometric methods have needed more efficient absorbers to permit the use of larger air samples. The latter need recently has been filled by the absorbers of MARTIN and GREEN (6), HEINICKE and HOFFMAN (4), and THOMAS (9). A unique method of determining carbon dioxide exchange has recently been devised by MCALISTER (7). After this paper was submitted for publication, a paper by WAUGH (10) appeared, describing a hand-operated conductivity appa-

<sup>1</sup> Grateful acknowledgment is made for assistance in carrying out these investigations to the following: Operation of apparatus: LYNN BROWN and J. ALLEN; agronomic observations: L. W. NIELSEN; calculation of results: T. R. COLLIER, D. BONNER, T. BUNKALL, M. CHRISTENSEN, F. EVANS, R. HIRST, S. R. IRVINE, and G. MINER; chemical studies: R. H. HENDRICKS, A. ANDERSON, R. EVANS, H. LINFORD, D. PETERSON, G. F. SOMERS, G. TANNER, and D. WILLIAMSON; records: M. R. BERNSTON.

ratus, based on the same principles as have been employed in the automatic apparatus used in this work. WAUGH refers to a recent paper by HOLDHEIDE, HUBER, and STOCKER,<sup>2</sup> who describe a somewhat similar apparatus.

### Description of apparatus

We have worked with six-foot square plots of plants growing in the field under as nearly natural conditions as possible. Over these plots is placed a celluloid-covered cabinet, through which measured volumes of air are passed at rates ranging up to several hundred cubic feet per minute. Continuous and simultaneous measurements of the carbon dioxide content of normal air going into the cabinet and coming from it are made.

### THE CO<sub>2</sub> AUTOMETER

The carbon dioxide analytical machine or autometer was described in 1933 in *Industrial and Engineering Chemistry* (9). A diagram of the essential features of this apparatus is presented in figure 1, which illustrates clearly the direction of flow of liquid and gas through the system under the control of the cam-operated automatic valves A to T, inclusive. The autometer has two absorbers, employing 0.005 N sodium hydroxide as absorbent. These absorbers are capable of scrubbing out all of the CO<sub>2</sub> from a stream of air passing at about 300 cc. per minute. This is accomplished by using a sintered glass disk to break up the air stream into very fine bubbles, and a surface tension depressant—normal butyl alcohol—in the liquid, to cause foaming and thus to prolong the period of contact between the bubbles and the liquid. The progress of the absorption is followed by measuring the electrical conductance of the solution at constant temperature with a recording Wheatstone bridge, making use of the fact that sodium hydroxide solution has about twice the conductance of the equivalent sodium carbonate solution.

A motor and reduction gear system, not shown in figure 1, operates an air-pump, and also the two camshafts indicated at the top of the figure. The cams open and close the automatic valves in proper sequence so that the aspiration of the absorbers alternates every two minutes, and the absorbing solution is retained for 8 successive aspirations in each absorber before being drained out and replaced with a fresh charge. The electrical conductance of the solution is automatically recorded each time the absorber is quiescent. An air sample of exactly 600 cc., or any other desired volume, is dispensed to each absorber by a special mercury gas meter in each 2-minute period. The meter consists of two flat steel boxes 15 × 20 × 2 cm. deep, connected through a U-tube. The meter contains enough mercury to fill one box com-

<sup>2</sup> HOLDHEIDE, W., HUBER, BR., and STOCKER, O. Eine Feldmethode zur Bestimmung der momentanen Assimilationsgrösse von Landpflanzen. Ber. d. bot. Ges. 54: 168–188. 1936.

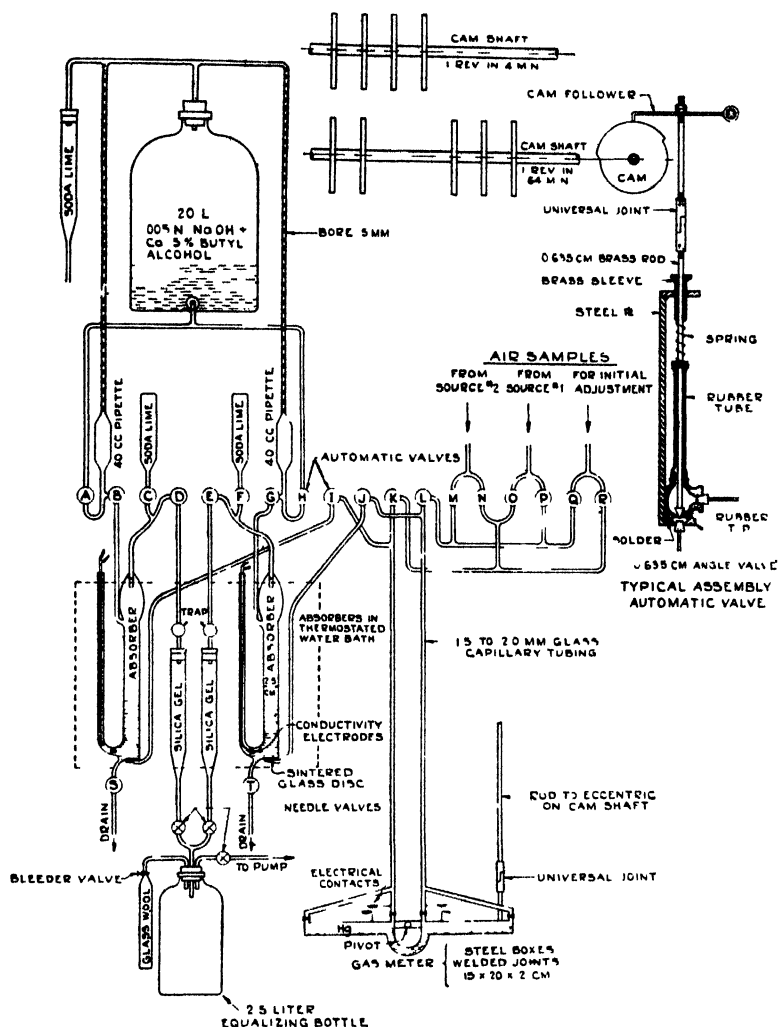


FIG. 1. Diagram of the carbon dioxide autometer. The method of measuring and controlling the flow of liquid and gas is shown.

pletely and leave 600 cc. of free space in the other. The mercury, under the control of valves, D, E, I, J, K, and L, flows alternately from one box to the other, thereby dispensing the air sample before it and drawing in a fresh sample behind it. An eccentric on the camshaft raises and lowers one side of the meter on a pivot so that there is never an appreciable hydrostatic head of mercury in either box. The rate of flow of the gas is adjusted by the suction system so that the entire gas sample is withdrawn from the meter in a few seconds less than the 2 minutes allotted for the operation. The

mercury is then drawn up into the glass capillary tubing above the meter a distance of about 5 cm., depending on the reduction of pressure in the equalizing bottle, which is controlled by the bleeder valve, until the end of the 2-minute period. The electrical contacts on the gas meter permit the registration on the recorder chart of the fact that the complete sample has been withdrawn.

A cycle consists of 16 2-minute periods, giving 8 samples to each absorber, which retains the same absorbing solution throughout the cycle. In the next cycle the absorbers are recharged with liquid and the source of the samples is reversed. This reversal is accomplished by the valves M, N, O, P, two of which are open while the other two are closed. The valves Q and R supply the first sample of air from a large reservoir for the initial adjustment of the solution. Thus by averaging two consecutive cycles any slight machine differences between the absorbers or electrodes can be canceled out and an accurate measure of the difference between the  $\text{CO}_2$  concentrations at the intake and outlet of the plant chamber over a 64-minute period can be obtained. This principle of alternating the source of the air samples is also employed when comparing two plant chambers using two autometers.

#### THE $\text{CO}_2$ RECORDER CHART

A section of the recorder chart is illustrated in figure 2. This chart represents the  $\text{CO}_2$  concentrations at the intake and the outlet of a plant chamber between the hours of 4:40 P.M. and 8:15 P.M. on August 6, 1936. The significance of the record may be understood when it is pointed out that A represents the conductance of the spent solution in the first absorber just before it is drained out, causing the indicated conductance to fall to zero. B represents the corresponding conductance of the second absorber. The conductance of the two fresh and initially-adjusted solutions are shown at C and D. Then the conductance decreases in steps after each pair of 2-minute absorptions until the cycle is completed at E and F. These solutions are drained out, the sources of the air samples reversed, and a new cycle commences at G and H. The track at the right margin proves that a full air sample was dispensed for each absorption. At the beginning of a number of the cycles in this chart the source of the samples for the individual absorbers—whether intake or outlet of the plant chamber—is designated. Since the volumes of the absorbing solution and the volumes of the air samples are constant, the displacement of the conductance of the solution from C to E and from D to F represent the concentration of the carbon dioxide in the intake and outlet air respectively. For each cycle, the intake and outlet concentrations have been calculated and placed upon the graph; also the percentage change of concentration of the air stream on passing through the plant chamber. Pairs of cycles have been grouped together and averaged

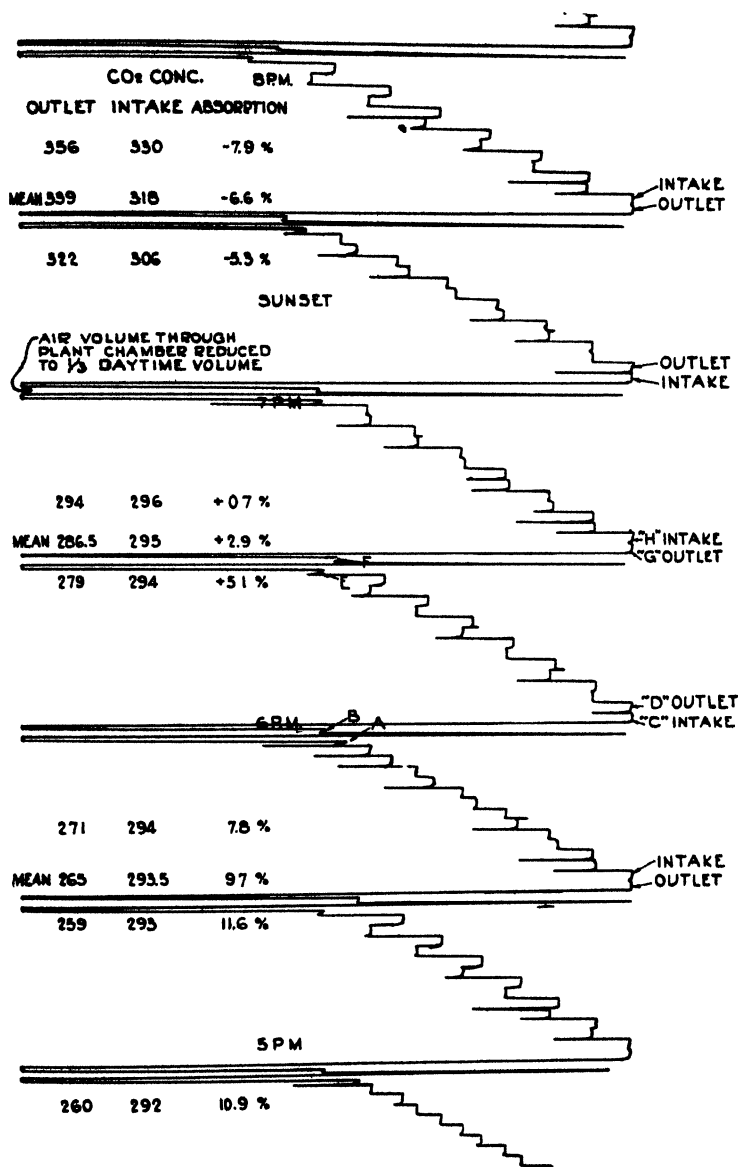


FIG. 2. Sample section of the recorder chart, made during the evening of August 6, 1936. The recorder gives the analyses of intake and outlet air samples from a plot of alfalfa. This chart indicates photosynthesis during sunlight (to 7:05 P.M.). Thereafter it indicates respiration.

to give the values for 64-minute periods. It will be noted that there is a decrease in the positive values of the absorption until 6:30-7:00 P.M., when



the two concentrations were almost equal, indicating a decrease in the amount of photosynthesis with the approach of sunset at 7:20 P.M. In fact, close scrutiny of the record reveals that the compensation point occurred at about 7:05 P.M. Thereafter the amount of carbon dioxide leaving the plant chamber was greater than that which entered, indicating respiration. The air flow through the plant chamber was reduced to one third its daytime value at 7:00 P.M. This accounts for the apparently exaggerated amount of respiration as compared with the earlier assimilation. It should be noted also that the concentration of carbon dioxide in the air rose from about 292 parts per million during the first hour depicted, to 330 parts per million in the last cycle. This rise in the concentration of  $\text{CO}_2$  in the air occurs regularly in the evening and the reverse effect is noted regularly in the morning. It is doubtless caused by the depletion of  $\text{CO}_2$  in the air close to the ground by photosynthesis in the daytime and its enrichment by respiration at night.

#### THE PLANT CHAMBER

Figure 3 is a photograph taken September 7, 1936, of a pair of the plant chambers numbered 7-A and 8. The  $\text{CO}_2$  exchange of these two plots, which appear to be identical in stand, is presented below. The photograph shows a blower and the 8-inch galvanized pipes leading into the chambers; also tin windows, which permit some access into the interior of the chambers, and the small glass sampling tubes through which samples of intake and outlet



FIG. 3. Plots 7-A and 8 on September 7, 1936, illustrating the plant chamber and blower.

air are taken to the autometers for carbon dioxide analysis. A celluloid window is shown on the intake pipe, through which an anemometer may be read without disturbing the air flow. The exit pipe is just beyond the intake pipe and it also contains an anemometer. These instruments are not removed from the pipes except to interchange them between the different pipes. They are read at frequent intervals and are interchanged daily.

A careful study has been made of the anemometer readings in order to convert the indicated velocity readings into air volumes. For this purpose it has been necessary to determine the channeling of the air in the pipe and also to determine the relations between the average rates of flow on a given cross-section and the volume of air passing through the pipe. These relations have been determined empirically by passing measured volumes of air through the pipes and measuring the velocities with anemometers under conditions simulating those which obtain in the experimental work. In this way it has been found that the pipe containing the anemometer behaves as if it were considerably smaller than its dimensions would indicate. Since the anemometers do not give a reliable indication at low air velocities the practice has been adopted of placing a rubber collar around the instrument, so as to force all of the air to go through it when small air volumes are used. In this way it is possible to measure small air volumes (1000 liters per minute or less) such as are used at night, with the same precision as the larger air volumes (4000 to 7000 liters per minute), which are employed during the day.

By measuring the volume of air entering and leaving the plant chamber it is possible to determine the leakage in the system. This seldom exceeds 10 per cent. of the total volume, and the air volume employed in the calculations is the mean of the intake and outlet volumes.

### Respiration and photosynthesis data

#### ALFALFA

The respiration and apparent photosynthesis values of two plots, nos. 7-A and 8, which were measured continuously and simultaneously for 26 days, from September 4 to October 1, 1936, except for 2 days when the equipment had to be used for other purposes, are illustrated in figure 4, charts A, B, and C. The abscissae represent time. The occurrence of 6 A.M. and 6 P.M. on the different days is indicated. The ordinates represent grams of carbon dioxide absorbed per period of 64 minutes. The values below the zero ordinate indicate respiration; above zero, apparent assimilation.

The symmetry of the curves is striking; also the fact that the independent, although similar, plots show such close concordance. Many pairs of points are coincident, though at times the curves depart from each other slightly.

Seventeen of the 26 days were cloudless and the photosynthesis curves are quite regular and symmetrical. Clouds of different types occur at least

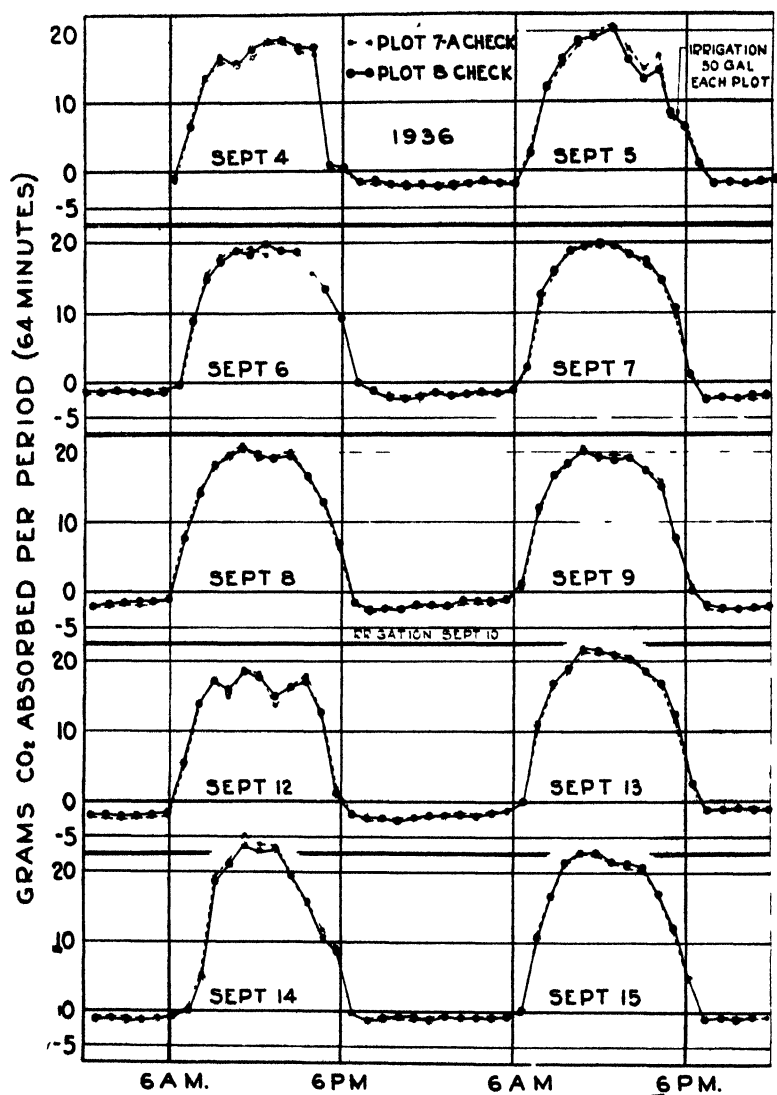


FIG. 4. Chart A. Photosynthesis and respiration curves for alfalfa plots 7-A and 8, from September 4 to September 15, 1936. The values for each 64-minute period of the respiration at night and apparent assimilation during the day on each plot are indicated.

at times on the other 9 days illustrated, and this cloudy weather is reflected by irregularities in the assimilation curves. For example, a very heavy cloud in the late afternoon of September 4 caused photosynthesis to drop almost to zero at 4:30 to 5:30 P.M., and dense clouds in the morning of September 14 delayed the beginning of photosynthesis over an hour. The irregularities

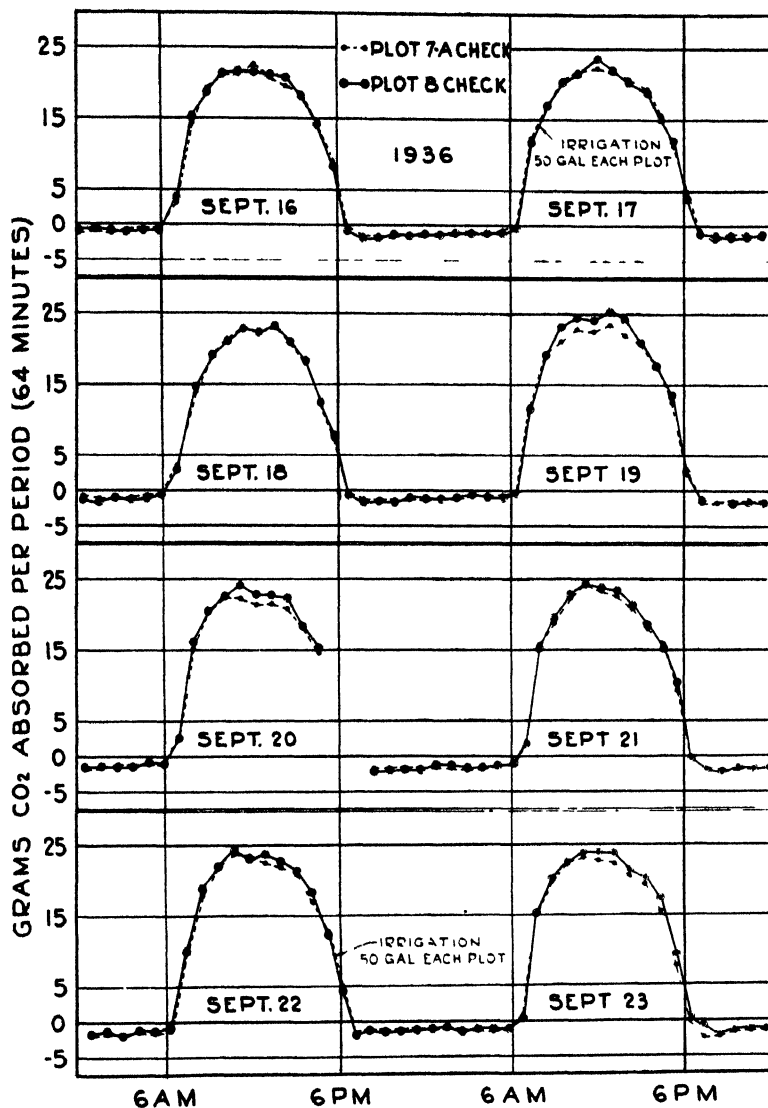


FIG. 4. Chart B. September 16 to September 23, 1936.

on September 5, 12, 25, 26, and 27 were due to intermittent clouds during these days. September 25 and 26 will be referred to again later.

There is a 5-day period between September 19 and 24 in which plot 7-A appears to be less active than plot 8 during part of the day. This condition we believe to be due to failure of irrigation water to penetrate plot 7-A uniformly, with the result that one side of the area was appreciably drier than

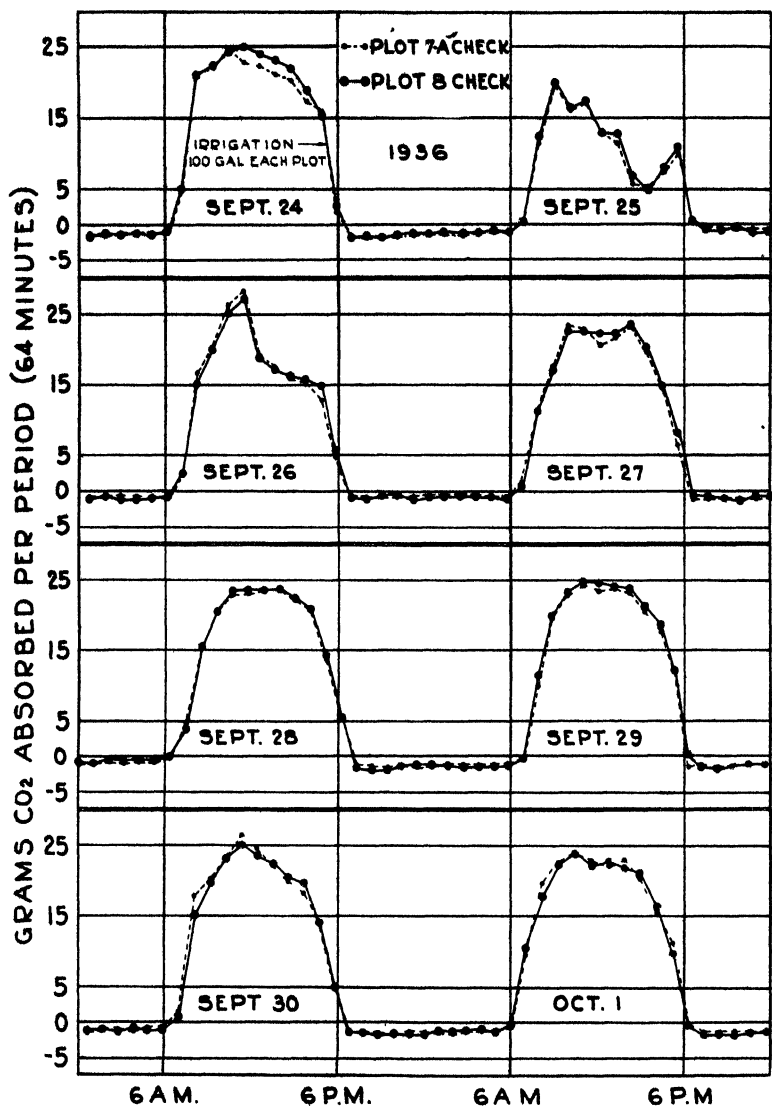


FIG. 4. Chart C. September 24 to October 1, 1936.

the rest. When this plot was heavily irrigated on September 24 the photosynthesis of the two plots quickly came to close agreement again.

The respiration curves are nearly coincident. It will be noted that there is a tendency for the amount of respiration to decrease as the night advances, owing to a lowering of the temperature. In this connection it should be pointed out that no account has been taken in this work of "soil respiration."

A number of observations have indicated that this is a second-order factor, but more work needs to be done on this point.

The data of plots 7-A and 8 are summarized in table I, which is a balance sheet of growth and carbon dioxide exchange. The table gives the total daily apparent assimilation, respiration, and net assimilation, and their ratios, for each day during the period of observation. The total net assimilation is also given. The net assimilation values have been converted into equivalent dry matter, assuming on the basis of chemical analysis that the dry plant substance contained 44 per cent. of carbon. An estimate was made of the amount of dry material on the plot on September 4, when the observations began, by harvesting adjacent areas of alfalfa which appeared to be equivalent in height and density of stand. The plots were harvested on October 2, and the dry matter was determined. In this way it is shown that only 16.4 per cent. to 16.7 per cent. of the total net assimilation could be accounted for as top growth. The remainder probably entered the roots and there was stored. Similar experiments carried out in the summer have accounted for much larger percentages of the carbon dioxide assimilated as top growth. Evidently the low temperatures obtaining in September were conducive to storage of the products of photosynthesis rather than to top growth.

It will be noted that the rate of net assimilation was 15 per cent. greater on September 29 than on September 6, which were comparable days. In this period the estimated increases in total functioning dry matter and leaves were 23 per cent. and 16 per cent. respectively.

#### WHEAT

A few experiments have been carried out with wheat. The variety of wheat used was Utah Experiment Station no. 11544, a very uniform pure-line selection from Hard Federation  $\times$  Dicklow—a cross made some years previously. The curves of photosynthesis and respiration are quite similar to those obtained with alfalfa. Figure 5 presents the total daily apparent assimilation of a plot of wheat which was under observation from June 6, when the plants were just entering the boot stage, until July 12, when the grain was in the dough stage. This plot consisted of 4 rows of plants with 20 plants in each row. Some indication of the light intensity on the different days is given by the use of letters, which indicate whether the day was cloudless or whether the clouds were light, medium, or dense. On July 8 one row of the plot was harvested to obtain material for analysis and also to establish the amount of plant substance on the plot. Accordingly, on the last 5 days the amount of apparent assimilation has been calculated to a quantity of plant substance comparable with the rest of the experiment, as indicated by the circled points. In spite of the daily fluctuations it is apparent from the chart that the assimilation level of the wheat increases to maximum value at the flowering stage, then decreases through the milk and dough stages.



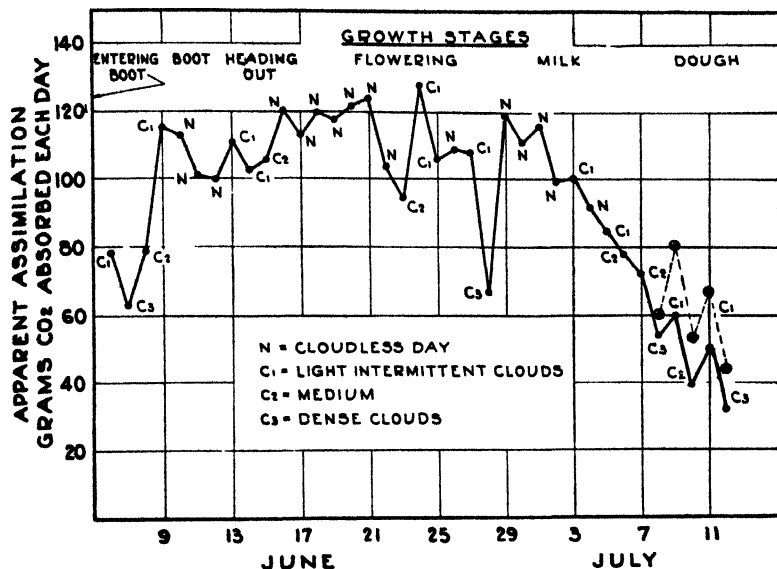


FIG. 5. Total daily carbon dioxide absorption of wheat from June 6 to July 12, 1936. The type of light conditions prevailing on the different days is shown. On July 8 one-fourth of the plot was harvested and the subsequent data have been adjusted to the original quantity of plant substance as indicated by the circled points.

When the experiment was discontinued the assimilation level was only about half of the level during the period of maximum activity. At this time the amount of functioning tissue was greatly curtailed due to the senescence. On July 8 55 per cent. of the leaf blades were dead, including 8 per cent. of the apical, 12 per cent. of the second from the top, and 37 per cent. of the third leaf blades.

Table II is a balance sheet of growth and carbon dioxide exchange on this plot of wheat. The data are presented in the same form as in table I. It will be observed that the apparent assimilation is considerably lower in the wheat plot than in the alfalfa plot. This is due largely to the fact that there was a considerably smaller amount of functioning tissue on the former. On the other hand, the respiration levels of the wheat and alfalfa are approximately the same, which is probably explained by the fact that the night temperatures during June were higher than the night temperatures in September. It is particularly interesting to note that 83.3 per cent. of the total carbon dioxide assimilated is accounted for as top growth, with only 16.7 per cent. which may be assumed to be root increment.

### Statistical analysis of the analytical data

The following statistical analysis will consider first the errors that may be expected in the absorption data as a result of errors attributable to the



TABLE II

BALANCE SHEET OF GROWTH AND CARBON DIOXIDE EXCHANGE PLOT 6 (WHEAT)

DATE	APPAR- ENT ASSIMI- LATION	NIGHT RESPIRA- TION	NET ASSIMI- LATION	DATE	APPAR- ENT ASSIMI- LATION	NIGHT RESPIRA- TION	NET ASSIMI- LATION
(1936)	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>		<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
June 6...	78.6	17.4	61.2	June 24...	128.4	20.5	107.9
" 7...	62.9	11.6	51.3	" 25...	106.8	21.3	85.5
" 8...	78.9	7.0	71.9	" 26...	109.0	26.0	83.0
" 9...	115.6	7.6	108.0	" 27...	108.6	14.3	94.3
" 10...	113.7	16.6	97.1	" 28...	67.6	16.8	50.8
" 11...	101.2	21.2	80.0	" 29...	119.6	17.5	102.1
" 12...	100.5	18.7	81.8	" 30...	111.2	21.1	90.1
" 13...	111.1	22.8	88.3	July 1...	116.8	17.6	99.2
" 14...	103.6	25.3	78.3	" 2...	99.7	25.6	74.1
" 15...	106.3	10.8	95.5	" 3...	100.9	22.6	78.3
" 16...	120.8	21.8	99.0	" 4...	92.3	14.2	78.1
" 17...	113.3	25.1	88.2	" 5...	85.3	21.7	63.6
" 18...	120.0	17.6	102.4	" 6...	81.2	20.4	60.8
" 19...	118.5	21.3	97.2	" 7...	72.8	17.4	55.4
" 20...	122.4	13.9	108.5	" 8...	60.0*	14.5*	45.5*
" 21...	124.4	25.0	99.4	" 9...	80.4*	9.7*	70.7*
" 22...	104.2	25.2	79.0	" 10...	52.9*	19.7*	33.2*
" 23...	95.0	23.4	71.6	" 11...	66.8*	20.3*	46.5*
				" 12...	43.8*	16.9*	26.9*
Total .....					3595.	690.	2905.
Total CO <sub>2</sub> assimilated June 6 to July 8 .....							2682.
Equivalent dry matter .....							1663.
Estimated dry matter less roots on July 8 .....							1744.
Estimated dry matter less roots on June 6 .....							359.
Top growth—June 6 to July 8 .....							1385.
Top growth—per cent. of total CO <sub>2</sub> assimilated .....							83.3
Probable root increment—per cent. of total CO <sub>2</sub> assimilated .....							16.7

\* Adjusted.

operation of the autometer; and second, deviations in the respiration and assimilation values of two plots measured simultaneously by two autometers. The latter deviations will reflect not only the analytical errors of the two machines, but also the errors of measurement of the air volumes passing through the plant chambers, as well as actual differences in the respiration and photosynthetic levels of the two plots. When the analytical machines were constructed an extensive test was made to determine the differences that might be expected in the carbon dioxide concentrations of the two absorbers when they were sampling from the same source. This extensive

experiment (9) indicated an average deviation between the two absorbers of 0.42 per cent. Since that time, some improvements have been made in the apparatus and the mean of over 300 samples of the same sort have indicated an average deviation between the absorbers of 0.25 per cent. This machine error represents about 1 per cent. of the maximum photosynthetic values as our experiments are ordinarily carried out.

The data in figure 4, charts A, B, and C, have been analyzed by recording the difference between the pairs of analyses. The average hourly deviations (without regard to sign) between the two plots, 7-A and 8, ranged on different days from 0.21 gm. to 1.05 gm. during the daytime and from 0.118 gm. to 0.315 gm. during the night. The average daytime value was 0.60 gm., or, if the 5 days when the drought conditions complicated the results are omitted, 0.52 gm., and the average nighttime value 0.18 gm. These deviations represent 1 per cent. to 5 per cent. of the total absorption during the daytime and 5 per cent. to 15 per cent. of the night respiration. If these deviations were entirely due to errors in analysis, their average values would represent machine absorption errors of 0.3 per cent. to 0.5 per cent. in the daytime and 0.7 per cent. at night, *i.e.*, values 1 to 3 times the normal machine error of 0.25 per cent. A similar statistical analysis of another pair of plots amply confirms the values found for plots 7-A and 8.

When the hourly deviations are analyzed by FISHER's method (3) of "t" it is found that the differences are significant on September 19, 20, 22, 23, and 24, when drought conditions probably complicated the results. On only two other days, namely, September 14 and September 29, are the differences significant, and on these two days the absolute values of the differences are negligibly small. Significant differences at night occurred on September 8, 9, 11, 21, and 28, but only on September 11 was the average deviation for the night greater than the average value (0.18 gm. CO<sub>2</sub>) mentioned above. Evidently these deviations are so small that this equipment should serve to measure extremely small experimental effects.

### Influence of light intensity on photosynthesis

The influence of variable light intensity due to clouds was strikingly shown on a number of days in figure 4, charts A, B, and C. Three of these days have been replotted in figure 6 above the curve representing the light intensity as determined with a 10-junction pyrliometer placed horizontally, and a recording voltmeter. Intermittent measurements of the light intensity with a Weston cell confirmed the values given by the pyrliometer. The values of the light intensity at normal incidence also have been calculated from the values on a horizontal surface. September 15 was a cloudless day, and the assimilation curves are essentially regular. Their shape is intermediate between the shapes of the light curves at normal incidence and on

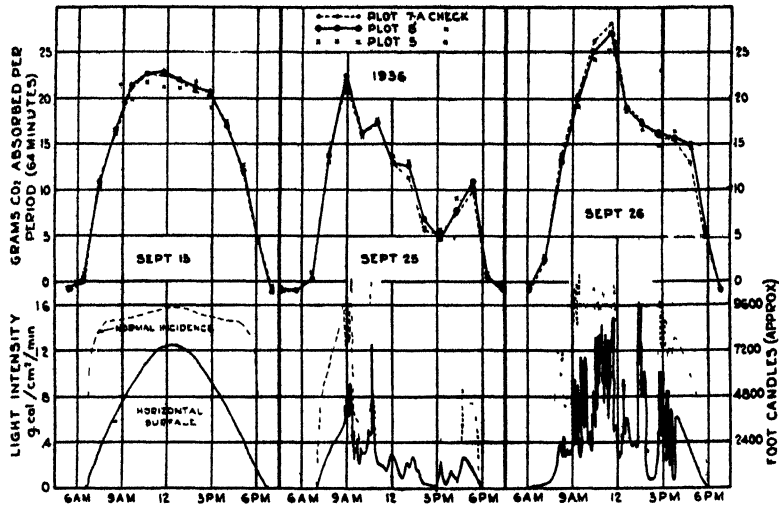


FIG. 6. Relation of photosynthesis to light intensity. Apparent assimilation of 3 different plots of alfalfa on September 15, 25, and 26, 1936, is plotted above the curves of light intensity on the same days. The curves illustrate the rôle of clouds in modifying the photosynthetic values.

the horizontal surface. September 25 and 26 were partly cloudy. It will be seen that the peaks of light intensity correspond closely with the peaks of photosynthesis. Of course, the method of averaging 64 minutes of record in calculating the points on the assimilation curve tends to smooth out irregularities due to too frequent changes in the light. For example, on September 26, a different grouping of the data would have shown a level equivalent to 10 grams between 1:30 and 2:40 P.M., and 20 grams between 2:40 and 3:10 P.M. The flash of sunlight between 1:20 and 1:30 P.M. can be distinguished in the record.

A striking relationship is illustrated on September 26. The photosynthetic maximum is appreciably higher than normal. This appears to be associated with the large fluctuations in light intensity due to frequent obscuring of the sun with clouds. It has frequently been noted that higher than normal apparent assimilation levels are reached when the sun is intermittently obscured by clouds for a few seconds to one or two minutes. This is probably due in part to a lower leaf temperature with attendant lower respiration. It may also be due in part to the orientation of the chloroplasts.

Incidentally it should be pointed out that in addition to the assimilation values of plots 7-A and 8, those for plot 5 also have been placed on the graphs as unconnected crosses. The concordance is excellent, not only in full light, but also in cloud shadow, and particularly so considering the fact that plot 5 is a different variety of alfalfa, and the plants are somewhat older.

Figure 7 illustrates some assimilation curves on cloudless days. The lower curve in the chart to the left represents a plot which is just starting

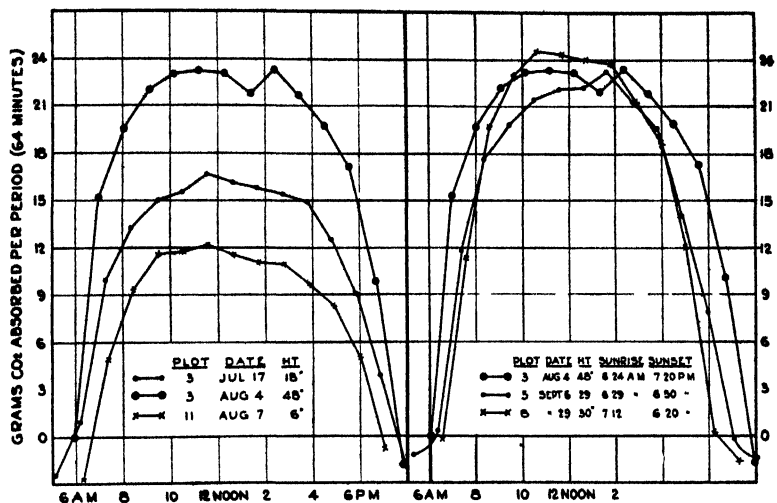


FIG. 7. Relation of stage of growth and length of day to photosynthesis. Apparent assimilation of 3 plots of alfalfa at 3 stages of growth in midsummer (left); and 3 plots of comparable stage of growth at monthly intervals (right).

to grow, the plants being 6–8 inches high. Part of the plants are shaded early in the morning and late in the evening by the base of the cabinet, so that the beginning of assimilation is retarded in the morning and its completion hastened in the afternoon. The upper curve represents a full-grown plot at about the same time, with the plants about four feet tall. The center curve represents the latter plot when the plants were 18 inches high. It is interesting to note that the assimilation level is only twice as great in the plot with plants 48 inches tall and having 3.6 times the weight of leaves, as in the plot with plants 6–8 inches tall, due, at least in part, to a larger proportion of shaded leaves under the fully illuminated surface leaves on the larger plot.

The chart on the right illustrates three more or less comparable plots at approximately monthly intervals. This chart shows the effect of the shorter day, but indicates about the same maximum apparent assimilation level regardless of the fact that the maximum angle of incidence of the sun on September 29 was  $47.5^\circ$  as compared with  $66.5^\circ$  on August 4. Indeed, the apparent assimilation level on the later date was somewhat higher than on earlier dates which was probably a reflection of a lower rate of respiration due to lower temperature.

In figure 8, the effect of clouds in reducing the assimilation level is illustrated. It will be seen that there is no appreciable reduction in the rate of assimilation as long as the light intensity exceeds about 52 per cent. of its

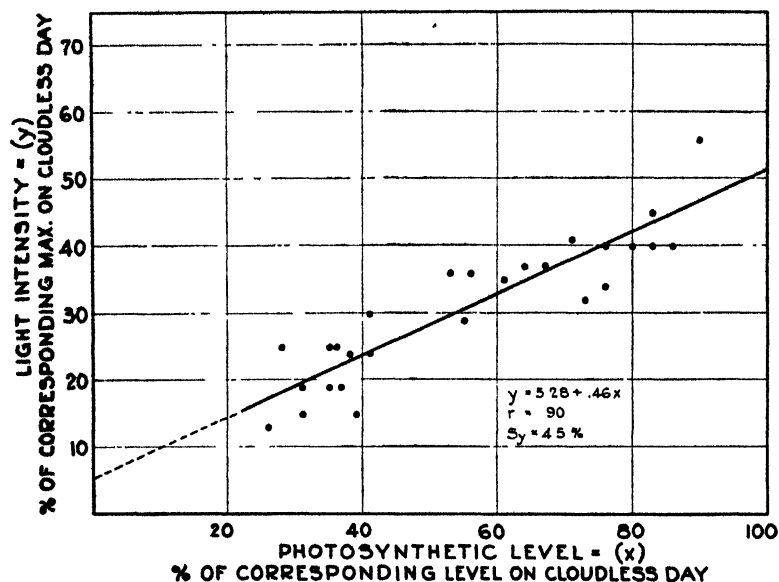


FIG. 8. Relation of photosynthesis to light intensity. The influence of clouds of different densities in reducing the photosynthetic level of alfalfa.

maximum value. The curve seems to indicate a linear relationship between the photosynthetic level and light intensities below 52 per cent. of the normal maximum at Logan, Utah.

### Effect of temperature on respiration

Figure 9 illustrates the effect of temperature on the respiration level and is derived by a consideration of the respiration values on a number of different nights in September. During this time there was very little top growth on the plot, and accordingly the quantity of vegetation is substantially constant over the period from which the respiration and temperature values were selected. It will be seen that the respiration level increases about fourfold with a temperature rise from zero degrees to 20° C., which is about the value expected from other work in the literature.

In a number of other experiments, temperature coefficients of approximately this value have also been obtained.

### Transpiration

It seemed desirable to attempt to measure transpiration, in addition to the carbon dioxide exchange in the plant chambers. Accordingly, during the summer of 1936, four units of the Leeds & Northrup relative humidity recorders (1) were purchased. This recorder employs a pair of carefully-balanced resistance thermometers, mounted in knife-shaped cases. One of

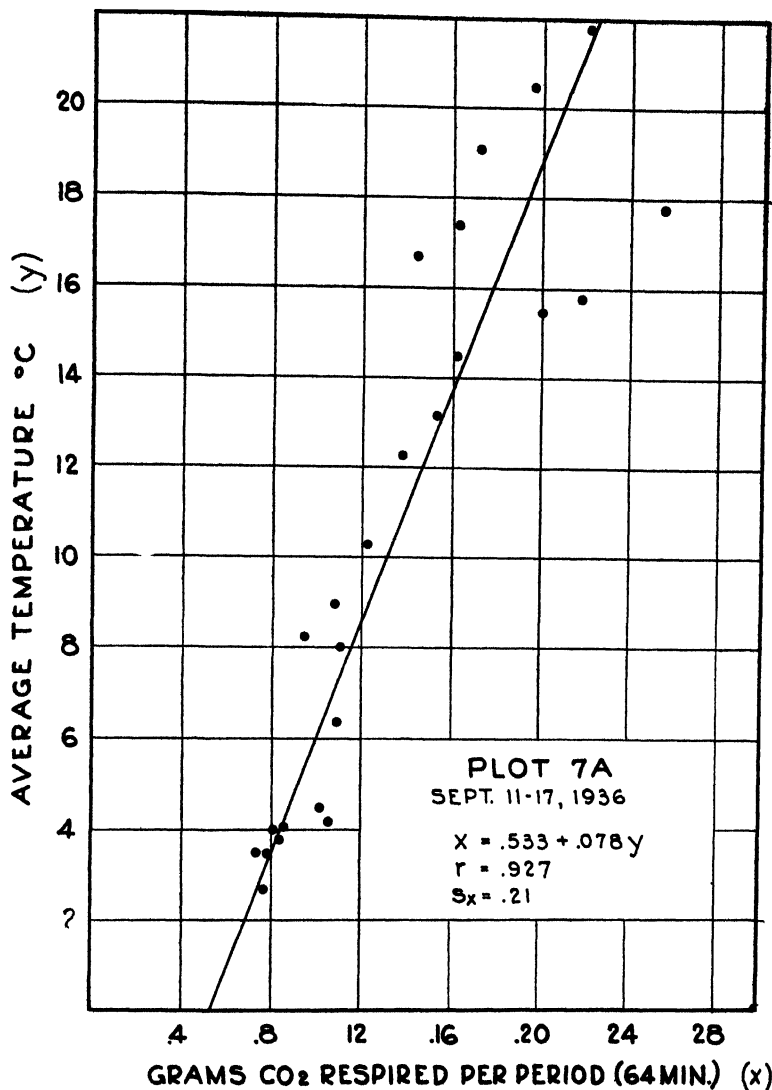


FIG. 9. Influence of temperature on the respiration of alfalfa.

the thermometers is wet by a wick. If wet-bulb temperature is plotted against dry-bulb temperature for a given value of the relative humidity, a straight line is obtained, the slope of which depends on the value of the relative humidity. Relative humidities from 20 per cent. to 100 per cent. thus yield a family of straight lines which on extrapolation intersect in a very limited area near a point corresponding to about  $-10^{\circ}\text{C}$ . The recorder measures the relative resistance of the wet- and dry-bulb resistance ther-

mometers from this point of reference. Thus the instrument reads relative humidity directly regardless of the temperature of the system. The instrument was calibrated by immersing the thermometers in baths of different temperatures and comparing the readings with the hygrometric tables. It was also checked by direct analysis of the air for water vapor. The manufacturer's claim, that the limit of error in the performance of the recorder is within 1.5 per cent. of relative humidity, was confirmed.

Units of the apparatus were set up in the intake and outlet pipes of the plant chamber, dampers being used to insure sufficient air flow over the wet bulb to produce maximum cooling. In addition, a group of resistance thermometers attached to an 8-point Leeds & Northrup temperature recorder made a continuous record of the air temperature at the points where relative humidity was measured.

In calculating the results, the temperature and relative humidity records were averaged by hours and the weight of water vapor which was carried past a given point was estimated on an hourly basis, using the vapor-pressure curve of water and the rate of air flow as determined by the anemometers in the pipes. Figure 10 is presented to illustrate the type of data which have

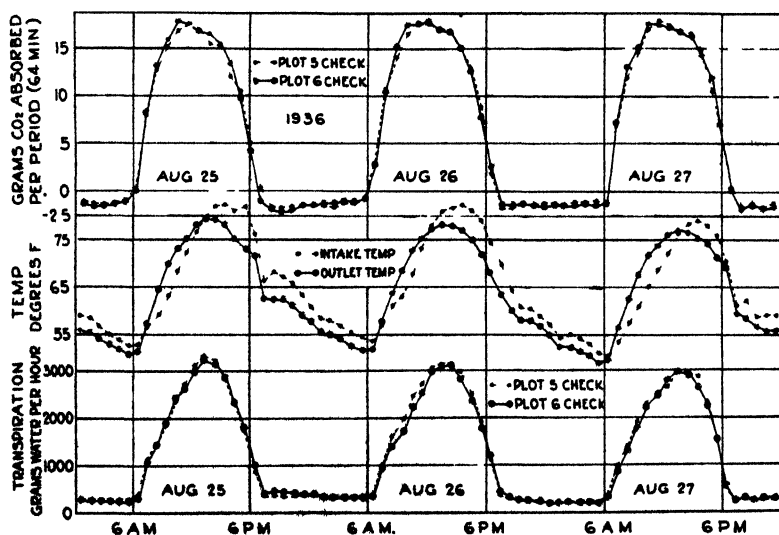


FIG. 10. Curves of respiration and apparent assimilation (upper); average intake and average outlet temperature (middle); and transpiration (lower) of two plots of alfalfa.

been obtained. The transpiration curves of two similar plots, plots 5 and 6, measured simultaneously, are given along with the corresponding carbon dioxide exchange curves and the average intake and outlet temperature curves on 3 cloudless days. The total amounts of water transpired on plots 5

and 6 were 31.1 and 31.3 kilos respectively on August 25; 30.8 and 29.4 kilos on August 26; and 28.2 and 27.6 kilos on August 27. The graphs and data indicate that transpiration can be measured continuously and automatically with an average deviation of less than 5 per cent. It is probable that the equipment is capable of better performance than this. The shapes of the transpiration curves are similar to those of the carbon dioxide curves, except that the former are more pointed and definitely skew, reaching their maximum on a cloudless day at about 2 P.M. There is obviously a close correlation between the rate of transpiration and the temperature, particularly during the day, when the temperature curves are also correspondingly skew. At night the response of transpiration to temperature is less noticeable, though definite. This is due to several causes: first, stomatal closure at night; second, reduction of the air volume passing through the plant chamber to 30 per cent. of the daytime volume; and third, lowering of both the vapor pressure of water and its temperature coefficient when the temperature is lowered. In any case, the graphs show clearly that both transpiration and respiration fall off progressively as the night advances along with the lowering of the temperature.

It should be noted that the outlet temperature exceeds the intake temperature during the morning, but during the afternoon and night the reverse is true. The cooling effect of transpiration is thus manifested at night, and also in the afternoon when evaporation removes more energy from the system than is added by solar radiation. In the morning, when the temperature is lower, the input of radiant energy exceeds the outgo through evaporation.

### Summary

1. This paper describes apparatus designed to measure continuously and automatically, the carbon dioxide exchange and the transpiration of plants growing under natural conditions.

2. The carbon dioxide analytical machine or autometer, with its most recent improvements, is briefly described, and a typical section of the recorder chart is presented to show how the apparatus operates.

3. This equipment has been used to measure simultaneously and continuously the carbon dioxide exchange on two similar plots of alfalfa over a period of 26 days. The hourly values of respiration and photosynthesis of the two plots are plotted. Data summarizing the daily apparent assimilation values of a plot of wheat covering a period from the boot stage to the dough stage are also presented.

4. The alfalfa curves show that two similar plots give closely concordant responses to the environment, both as to respiration and photosynthesis. The two curves are analyzed statistically, and it is evident that the apparatus may be used to measure very small effects of an experimental treatment.



5. The influence of clouds on rate of photosynthesis is strikingly shown by the curves. Further, data have been collected which indicate that the rate of photosynthesis of alfalfa is a linear function of the sunlight intensity up to about 52 per cent. of the normal maximum at Logan, Utah. Greater intensities do not increase the rate appreciably.

6. Respiration varies with the temperature, and increases about fourfold in rate between 0° C. and 20° C.

7. The balance sheet of the alfalfa plots shows that only 16.5 per cent. of the net carbon dioxide assimilated could be accounted for as top growth. The remainder was probably stored in the roots. This was a late-season experiment. In the summer a much larger proportion goes into the tops.

8. The wheat data show that the rate of assimilation reaches a maximum in the flowering stage, then falls off in the milk and dough stages owing to the senescence of the plant. The balance sheet accounts for 83.3 per cent. of the net assimilation as top growth.

9. Transpiration has been measured continuously and automatically using Leeds & Northrup relative humidity recorders. The transpiration curves resemble the carbon dioxide exchange curves in shape, but they are more pointed than the latter and also are definitely skew in the daytime. The transpiration curves evidently reflect temperature changes closely; whereas photosynthesis follows more nearly the light intensity. The average deviation of the transpiration values on two similar alfalfa plots was less than 5 per cent.

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# RELATION OF SULPHUR DIOXIDE IN THE ATMOSPHERE TO PHOTOSYNTHESIS AND RESPIRATION OF ALFALFA<sup>1</sup>

MOYER D. THOMAS AND GEO. R. HILL

(WITH TWELVE FIGURES)

## Introduction

One of the most fundamental processes in the growth of vegetation is that of photosynthesis, by which the principal portion of the plant substance is synthesized. This process involves delicate and complicated chemical reactions under the influence of light—reactions which are readily affected by external conditions. A study of the photosynthetic processes, and also of respiration, in connection with sulphur dioxide fumigations, should throw a great deal of light on the nature of the reactions of the gas with the vegetation.

A carbon dioxide analytical machine or autometer has been made (6), which is capable of measuring continuously and automatically the concentration of carbon dioxide at two points in an air stream. The use of this autometer in connection with a celluloid-covered plant chamber (2, 8) permits the determination of the carbon dioxide content of a measured volume of air as it enters and as it leaves the plant chamber, thus measuring the rate of photosynthesis during the day of the plants in the cabinet, growing under field conditions, and their respiration at night. By using two units of this equipment, a sulphur dioxide fumigation treatment has been applied to one and the effect of the treatment has been determined by comparing the rate of photosynthesis and respiration of the treated plot with that of a similar check plot.

This paper presents the complete carbon dioxide exchange history of 9 fumigated plots and their corresponding check plots, covering in each case the fumigation period and also periods preceding and following the fumigation. Experimental treatments have been studied ranging from fumigations which produced almost complete foliar destruction, progressively downward to long-continued sublethal dosages of the gas. The experiments are arranged in order of decreasing concentration of applied sulphur

<sup>1</sup> Grateful acknowledgement is made for assistance in carrying out these investigations to the following individuals: Operation of apparatus, LYNN BROWN and J. ALLEN; agronomic observations, L. W. NIELSEN; calculation of results, T. R. COLLIER, D. BONNER, T. BUNKALL, M. CHRISTENSEN, F. EVANS, R. HIRST, S. R. IRVINE, and G. MINER; chemical studies, R. H. HENDRICKS, A. ANDERSON, R. EVANS, H. LINFORD, D. PETERSON, G. F. SOMERS, G. TANNER, and D. WILLIAMSON; records, M. R. BEERTSON.

All of the chlorophyll analyses, and most of the sulphur analyses in this paper were carried out by Dr. R. H. HENDRICKS.

dioxide. The paper also includes the fumigation and yield data of these plots, together with sulphur and chlorophyll analyses of the plants. Other chemical and agronomic data on these plots, and also transpiration studies, will be reported later.

An earlier paper (2) compared the effect of leaf destruction by sulphur dioxide fumigation and by clipping on the yield of alfalfa. It was shown that the yield was reduced in proportion to the amount of leaf destruction and that if there was no leaf destruction there was no significant reduction in yield. The two methods of causing leaf destruction—sulphur dioxide fumigation, and clipping off the leaves with shears—produced nearly identical reductions in yield. A second paper (7) showed that the quantity of leaf destruction was proportional to the amount of gas absorbed by the leaves, and that an appreciable amount of sulphur dioxide, approximating one quarter of the amount necessary for complete leaf destruction, could be absorbed without any leaf destruction at all. It was also shown that the amount that could be absorbed without injury increased greatly as the rate of absorption decreased, due probably to the rapid conversion of absorbed sulphur dioxide to the thirty-or-more times less toxic sulphate form. The papers cited also described the apparatus and technique for conducting the sulphur dioxide fumigations. Briefly, the procedure consisted in adding the sulphur dioxide from a bottle of the liquid through a capillary flowmeter system to the air stream entering the plant chamber. The air stream was analyzed for sulphur dioxide continuously and automatically (5) as it entered and as it left the plant chamber, thus permitting the determination of the quantity of the gas absorbed by the vegetation. In addition, a record was kept of the environmental conditions—light, temperature, and relative humidity. Observations on growth, yield, and leaf destruction were also made.

As a matter of interest, in the charts which will be presented later, the occurrence of clouds has been indicated in a number of cases. It should also be noted that the carbon dioxide exchange data of 4 plots on the same days furnish many examples of quadruplicate records. Attention may be directed to August 23 and 28, 1935, on figures 10 and 12; to August 5, 1936, on figures 3 and 4; and to August 12, 14, and 16, on figures 4 and 5, as striking examples of these quadruplicate records.

### Effect of sulphur dioxide on alfalfa as indicated by the carbon dioxide exchange

#### I. EXPERIMENTS AT SALT LAKE CITY, UTAH

##### A. EFFECTS OF FUMIGATIONS PRODUCING NEARLY COMPLETE DEFOLIATION.—

At the Agricultural Research Laboratory of the American Smelting and Refining Company at Salt Lake City, Utah, two plots of approximately

equal stand were covered with the celluloid cabinets, and their photosynthesis and respiration were measured for several days in July, 1933. Then from 1:15 to 2:00 P.M. on July 17 one of the plots, no. 4—14, was given a 45-minute fumigation. The average sulphur dioxide concentration at intake was 7.0 p.p.m. (maximum 8.6 p.p.m.) and the average outlet concentration was 4.0 p.p.m. (maximum 5.1 p.p.m.). This treatment caused about 95 per cent. acute leaf destruction. The carbon dioxide exchange data of this plot and its check are presented in figure 1. The abscissa represents time, and the ordinate represents grams of carbon dioxide absorbed in each 64-minute period. Positive values indicate photosynthesis, negative values, respiration. It will be seen that within an hour, the fumigation caused the plot to cease assimilating carbon dioxide and actually to commence evolving carbon dioxide as if the plot were in the dark. On the next two days assimilation and respiration practically balanced each other, but for a few hours each day there was a small amount of assimilation. Thereafter, with the gradual appearance of new leaves, the plot increased in ability to assimilate, until on the ninth day the activity was about 65 per cent. of the check; and, allowing for the fact that before the fumigation the plot was 15 per cent. less active than the check, the recovery on this day was about 75 per cent.

Observation of plot 4—14 indicated that on July 19, 90 per cent. to 95 per cent. of the leaf tissue had been destroyed. Some of the lower leaves in the center of the plot were uninjured. On July 21 new leaves were seen to be forming, and dry leaves were falling off. On July 26 the plots were harvested. At this time 23 per cent. of the dry weight of the plants consisted of normal green leaves, 7 per cent. of bleached leaves, and 70 per cent. of stems. In addition, 135 grams of leaves were shed, representing 10 per cent. of harvest weight. The check plot had 35 per cent. of leaves. The sum of the percentages of green, marked, and shed leaves on the treated plot was thus greater than the percentage of leaves on the check plot, indicating a much more rapid growth of leaves on the treated plot, subsequent to the fumigation, than on the check.

The decrease in total assimilation in the period following the fumigation represented 35 per cent. of the harvest weight of the check plot, making allowance for the fact that the check plot was 15 per cent. more active than the treated plot for two days before the fumigation. The harvest weights of these crops, which grew from June 16 to July 26, were 1530 grams for the check and 1300 grams (plus 135 grams of dead leaves which had been shed) for the treated plot. The observed decrease in yield of 15 per cent. was only about half the decrease in net assimilation because, at this stage of growth, less than half of the assimilated carbon dioxide was utilized for top growth, the remainder probably going into the roots.

The experiment described above was the first that was carried out with two units of the carbon dioxide apparatus. The results obtained, while con-

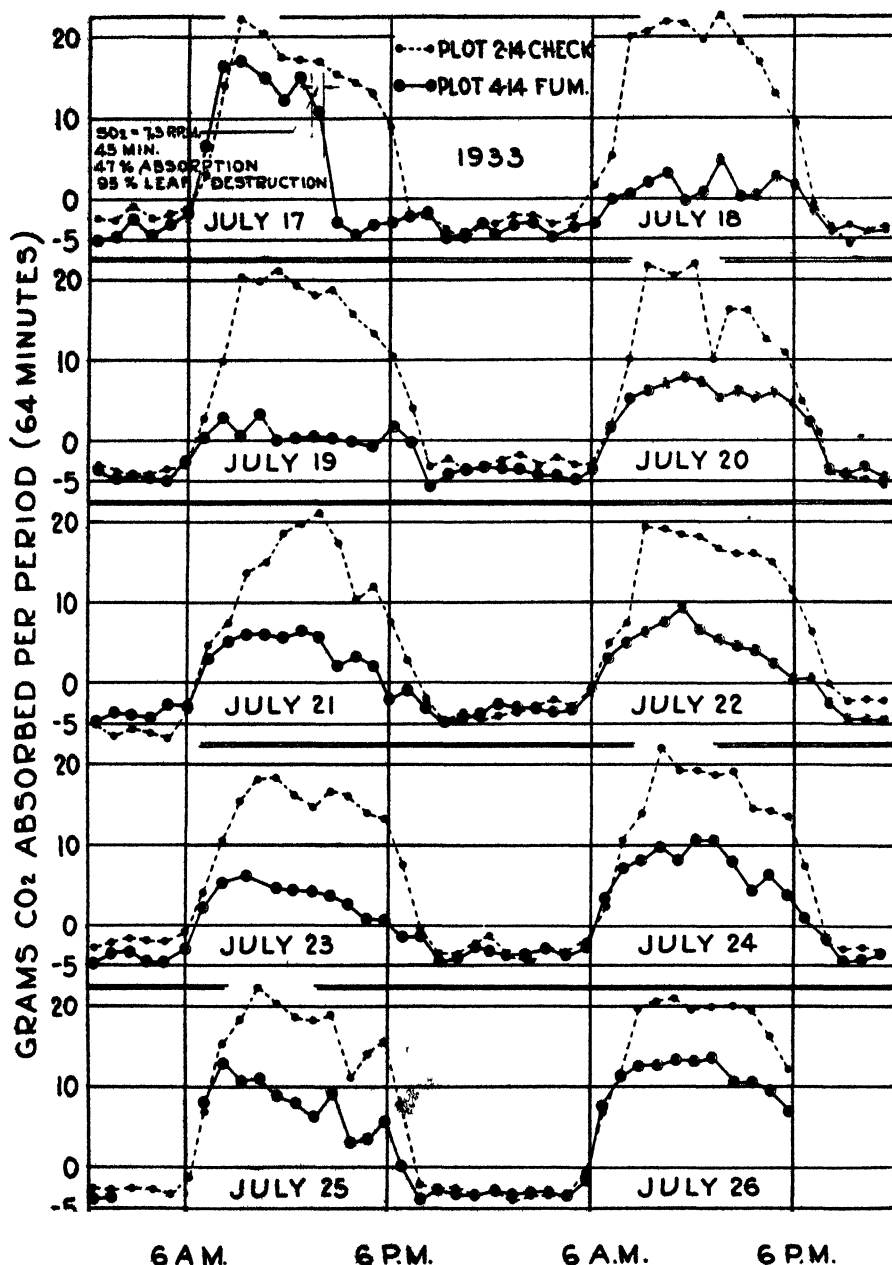


FIG. 1. Apparent assimilation and respiration of plots 2-14 and 4-14 (1933). Plot 4-14 was almost completely defoliated on July 17 by a sulphur dioxide fumigation. The curves show the subsequent recovery of the photosynthetic functions with the growth of new leaves.

clusive, were less accurate than later experiments, owing to initial difficulties with the machines. Another similar experiment will, therefore, be referred to briefly which confirms the results obtained with plot 4-14.

At 11:45 A.M. on September 1, 1933, a plot was accidentally fumigated with a concentration of 50 p.p.m.  $\text{SO}_2$  for nearly 3 minutes, which also caused about 95 per cent. leaf destruction. The photosynthetic activity of this plot declined gradually to the compensation point over a period of 2 hours. The carbon dioxide exchange did not become negative until evening. Thereafter the behavior of the fumigated plot with respect to the check was similar to the behavior depicted in figure 1. During the 3 days following the fumigation, apparent assimilation was about 7 per cent. of the check; then the level of activity began to rise. In 10 days, the level was about 65 per cent. of the check, and in 15 days about 80 per cent. At this time the plot was harvested. The dry weight of the plants consisted of 24 per cent. of green leaves, 12 per cent. of marked leaves, and 64 per cent. of stems. The check plot had 36 per cent. of green leaves and 64 per cent. of stems.

B. EFFECT OF SHORT FUMIGATIONS WITH HIGH CONCENTRATIONS OF SULPHUR DIOXIDE.—In the remainder of the experiments described in this paper, an attempt has been made to avoid producing an appreciable amount of leaf destruction. The next experiment to be described extended from August 1 to 11, 1933. The plots were third-crop alfalfa, which had been cut for the first crop on June 12, and for the second on July 12. Fumigation data for plot 2-13, which was treated on August 4, 7, and 10, are presented in table I. The data include the time and duration of the fumigations, air volumes and sulphur dioxide analyses, quantity and rate of absorption of the gas by the plants, and the extent of leaf destruction.

Carbon dioxide exchange data for plot 2-13 and its check are presented in detail in figure 2, and in summarized form in table II. It will be noted that on August 2 and 3 the apparent assimilation of the two plots was nearly identical. The data on August 1 were incomplete. At 10:30 A.M. on August 4 plot 2-13 was fumigated for 12 minutes, the sulphur dioxide being 8.5 p.p.m. at the intake and 4.0 p.p.m. at the outlet. At the first sign of injury to the leaves the fumigation was stopped. Actually 2.9 per cent. of the leaf area was destroyed. The effect of the treatment on photosynthesis was immediate, and during the hour following the fumigation the photosynthetic level fell to one third of its previous value; then the rate began to increase, but it was still subnormal at the end of the day. No effect was evident on the respiration level the following night. The photosynthetic level of the treated plot was 77 per cent. of the level of the check plot on August 5, and 87 per cent. on August 6. Recovery was complete on August 7. In fact the photosynthetic activity of the treated plot exceeded that of the check about 10 per cent. on both August 7 and 8.



TABLE I

FUMIGATION DATA OF PLOT 2-13-1933

DATE (1933)	FUMIGATION		AIR VELOCITY	CONCENTRATION OF SO <sub>2</sub>		SO <sub>2</sub> ABSORBED	LEAF DESTRUCTION (PERCENTAGE OF TOTAL LEAF AREA)
	STARTED	DURA- TION		INTAKE	OUTLET		
		<i>min.</i>	<i>l.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>gm.</i>	<i>rate*</i> %
Aug. 4	10:30 A.M.	12	3300	8.5	4.0	0.40	85.0
" 7	4:50 P.M.	78	4300	1.0	0.7	0.22	6.6
" 10	11:00 A.M.	70	4250	1.9	1.0	0.59	19.0
Total weight of SO <sub>2</sub> absorbed (grams)							1.21
Total weight of S absorbed (grams)							0.605

\* Milligrams SO<sub>2</sub> absorbed per minute per 1000 gm. dry leaves. Allowance made for 10 per cent. absorption by cabinet and soil, and for 5 per cent. absorption by stems, in all calculations of "rate of SO<sub>2</sub> absorption" in tables I, III, VI, IX, XII, XVI, XIX, and XXIII.

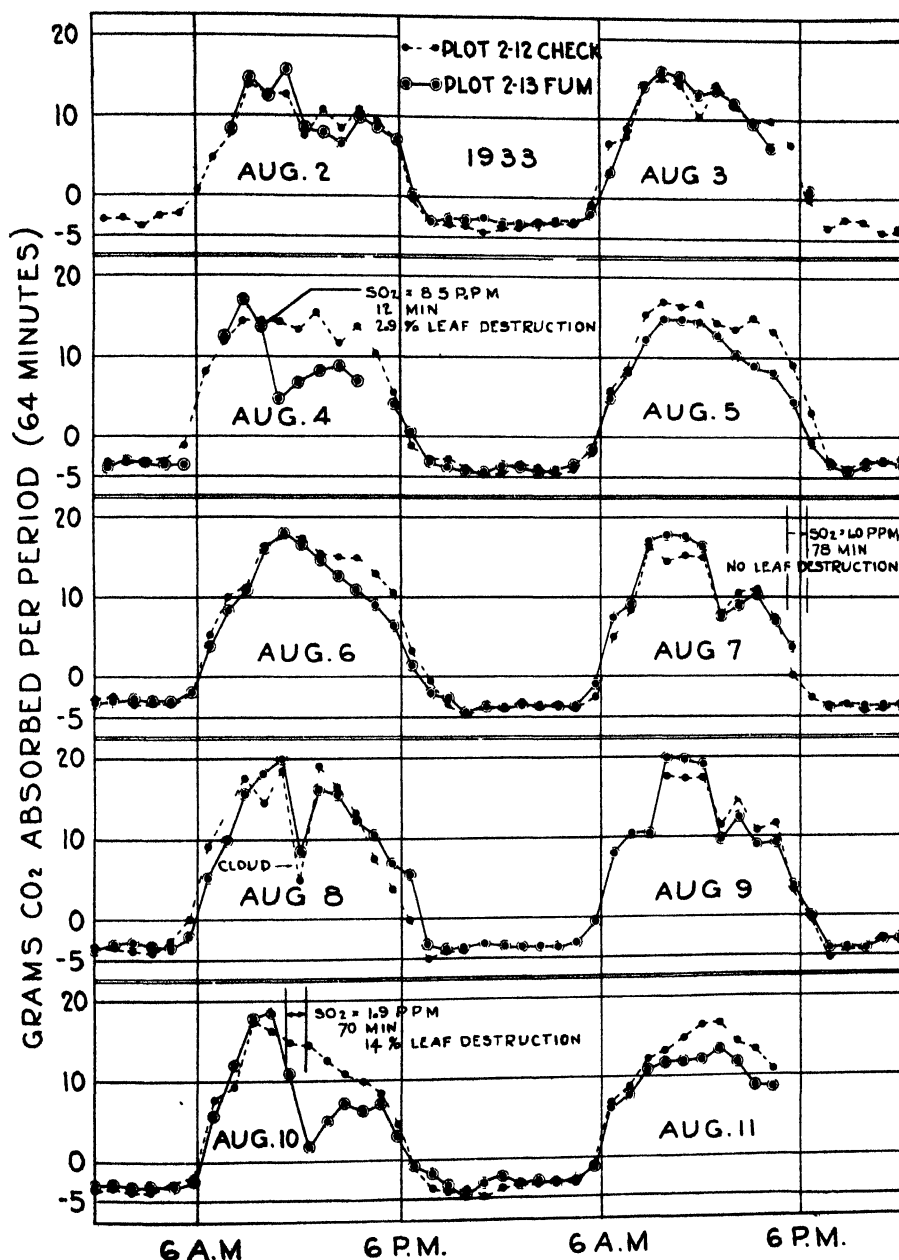


FIG. 2. Apparent assimilation and respiration of plots 2-12 and 2-13 (1933). Plot 2-13 was fumigated on August 4, 7, and 10 with high concentrations of sulphur dioxide but without causing extensive leaf destruction. The effect of the treatments on subsequent photosynthesis is shown.



A 78-minute fumigation with 1.0 p.p.m. of sulphur dioxide at the intake and 0.7 p.p.m. at the outlet, commencing at 4:50 P.M. on August 7, caused no leaf destruction and did not influence measurably the course of the carbon dioxide curves.

Beginning at 11 A.M. on August 10 a 70-minute fumigation, with 1.9 p.p.m.  $\text{SO}_2$  at intake and 1.0 p.p.m. at outlet, was applied, destroying 14 per cent. of the leaf area. This treatment was somewhat more drastic than the August 4 fumigation. Table I indicates the absorption of 0.59 gm. of  $\text{SO}_2$  as compared with 0.40 gm. on August 4, though the rate of absorption of the latter is much more rapid than the former. Apparent assimilation fell at once to 12 per cent. of its former value, and then began to rise in the second hour after the treatment. The level on August 11 was 81 per cent. of the check, but the experiment was unfortunately stopped before recovery from the treatment was complete. The curves on August 10 and 11 were quite similar to those on August 4 and 5, and it is probable that complete recovery would have occurred in about the same way as before, if the plots had not been harvested so soon, though the leaf destruction on August 10 would have delayed recovery slightly. Similar effects have been observed in a number of other experiments, and it appears certain that fumigations of the order of severity indicated, cause immediate interference with photosynthesis, followed by a rapid recovery of the function if only a small amount of leaf destruction has taken place.

The decrease in net assimilation due to the fumigations represented 6.2 per cent. of the yield of the check plot. Since only 76 per cent. of the net assimilation was converted into top growth, the calculated decrease in the latter due to the fumigations was 4.7 per cent. These values are somewhat low because recovery of the treated plot was not complete when the experiment was discontinued. They afford satisfactory confirmation, within the experimental errors involved, of the equation in an earlier paper (2, p. 234, equation 2) for two fumigations of a single crop producing an average of 8.5 per cent. leaf destruction. Equation 2 indicates a reduction in yield of 8.7 per cent., or if the origin of equation 2 is shifted to 100 per cent. the reduction would be 4.2 per cent.

Chemical analyses of representative samples of the two plots before and after the experiment showed an increment in sulphur content of the plants, 0.55 gm. greater in the case of the treated plot than the check, as compared with 0.605 gm. of sulphur added to the treated plot as shown by the gas analyses in table I. The 9 per cent. difference probably represented absorption of sulphur dioxide by the walls of the plant chamber and the soil.

## II. EXPERIMENTS AT LOGAN, UTAH

Preliminary experiments with concentrations of less than 1 p.p.m. were carried out at the Salt Lake City laboratory in 1933 and 1934. In order to

TABLE III  
FUMIGATION DATA OF PLOT 4—1936

DATE (1936)	FUMIGATION		AIR VELOCITY	CONCENTRATION OF SO <sub>2</sub>		SO <sub>2</sub> ABSORBED			LEAF DESTRUCTION	
	STARTED	DURA- TION		INTAKE	OUTLET	%	gm.	rate*	ACUTE	CHLOROTIC
July 21 ...	9: 26 A.M.	min.	l.p.m.	p.p.m.	p.p.m.	%	gm.	rate*	%	%
" 23 .....	8: 47 A.M.	170	3250	0.690	0.133	80.7	0.677	7.7	0.1	
" 25 ..	10: 08 A.M.	159	3680	0.656	0.141	78.5	0.665	8.1	0.1	
" 28 ..	12: 35 P.M.	64	4125	0.811	0.140	82.7	0.390	11.0	Traces	2.0
" 29 ..	11: 16 A.M.	60	4130	0.840	0.192	77.2	0.354	10.2	Traces	
" 31 ...	10: 11 A.M.	14	5820	0.900	0.171	81.0	0.121	11.0	0.5	5.0
August 3 .....	7: 00 P.M.	253	6050	1.26	0.826	15.5	0.140 <sup>a</sup>	...	...	
" 5 <sup>b</sup> ...				0.978			0.511	3.0	12.0	14.0
" 8 <sup>c</sup> ...									3.0	11.0
Duration of fumigations (hours) .....										12.3
Total weight of SO <sub>2</sub> absorbed (grams) .....										2.86
Total weight of S absorbed (grams) ..										1.43

\* Milligrams SO<sub>2</sub> absorbed per minute per 1000 gm. dry leaves.

<sup>a</sup> Estimated.

<sup>b</sup> From small sample.

<sup>c</sup> From entire harvest. Three per cent. weather discolorations on plot 3.

develop this phase of the problem in a place where sulphur dioxide would always be absent from the air, arrangements were made to work at the Agricultural College at Logan, Utah, during the seasons of 1935 and 1936. Nine 20 by 20-foot plantings of one-year-old alfalfa, belonging to the Botany Department of the College, were available. The areas represented a number of varieties of alfalfa. All exhibited vigorous growth and were uniform in stand. It was possible to place two plant chambers on an area, one to be treated, the other to serve as a check, and to have additional comparable material growing outside the plant chambers under entirely natural conditions.

C. EFFECT OF A SERIES OF FUMIGATIONS WITH 0.7 TO 1.26 P.P.M.  $\text{SO}_2$ .—Plots no. 3 and no. 4 were studied from July 15 to August 6, 1936. The alfalfa was Turkestan Selection no. 19,301. The fumigation data for plot 4 are presented in table III, which gives the time and duration of each treatment, together with the concentrations of sulphur dioxide applied and the amount and rate of absorption of the sulphur dioxide by the vegetation. The effect of these treatments, as measured by the carbon dioxide exchange, is shown in figure 3, charts A and B, and also in table IV, which summarizes the relations of the fumigated and check plots, and gives the relative values of the photosynthetic levels before, during, and after each fumigation.

A fumigation of 2.83 hours, with 0.69 p.p.m. on the morning of July 21 caused the photosynthetic activity to fall 18 per cent. during the time the gas was applied, but one hour after the fumigation was discontinued the activity was normal. A similar result was obtained on July 23. On July 25 a fumigation of 1.07 hours, with 0.81 p.p.m. at 11 A.M., caused the photosynthetic activity to fall about 44 per cent. during the hour that the treatment was applied. About an hour thereafter the plot came back completely to normal. A similar treatment at noon on July 28 caused a lowering of photosynthesis of about 25 per cent. The afternoon hours following this treatment were very cloudy, and the photosynthetic levels of the two plots were too low for differences to be significant. On the 29th, a fumigation of 18 minutes with 0.90 p.p.m. of sulphur dioxide lowered the photosynthetic rate about 13 per cent. for one hour and thereafter the plot returned to normal. A similar result was obtained on July 31, with 14-minutes application of 1.26 p.p.m. None of these treatments produced more than 1.0 per cent. of leaf destruction.

In the evening of August 3, a 4-hour fumigation with 0.98 p.p.m. was applied from 7 to 11:13 P.M. While the gas was present, the rate of respiration of the treated plot was only 62 per cent. of the check. During the remainder of the night the ratio was 120 per cent., which was about the normal ratio for the respiration of plots 3 and 4. Respiration appeared

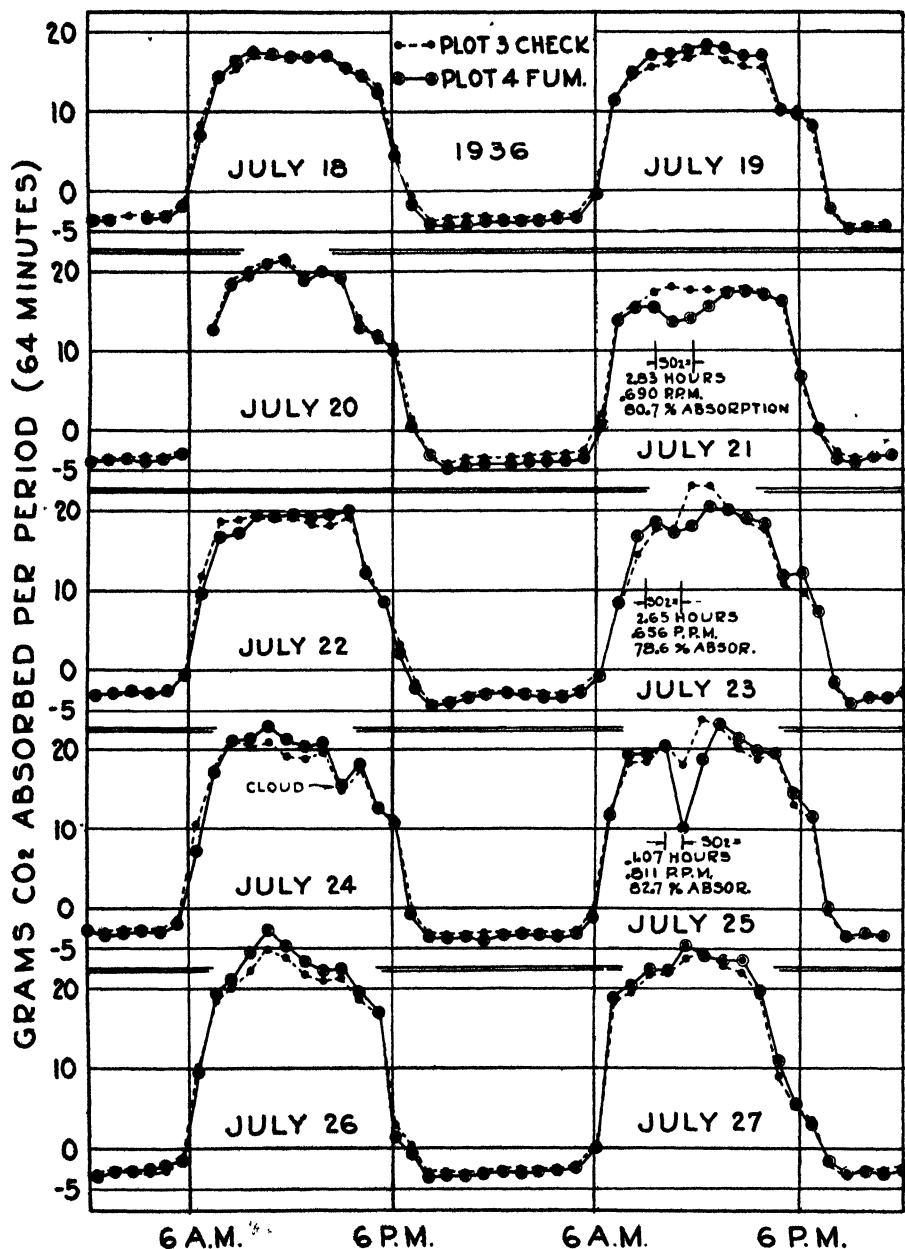


FIG. 3. Chart A, July 18-27, 1936. Apparent assimilation and respiration of plots 3 and 4 at Logan, Utah. The curves show the depressing effect on photosynthesis during the treatment of plot 4 of seven short fumigations with 0.7 to 1.26 p.p.m. SO<sub>2</sub>, together with the rapid recovery of a normal or greater than normal photosynthetic level when the treatment was discontinued.

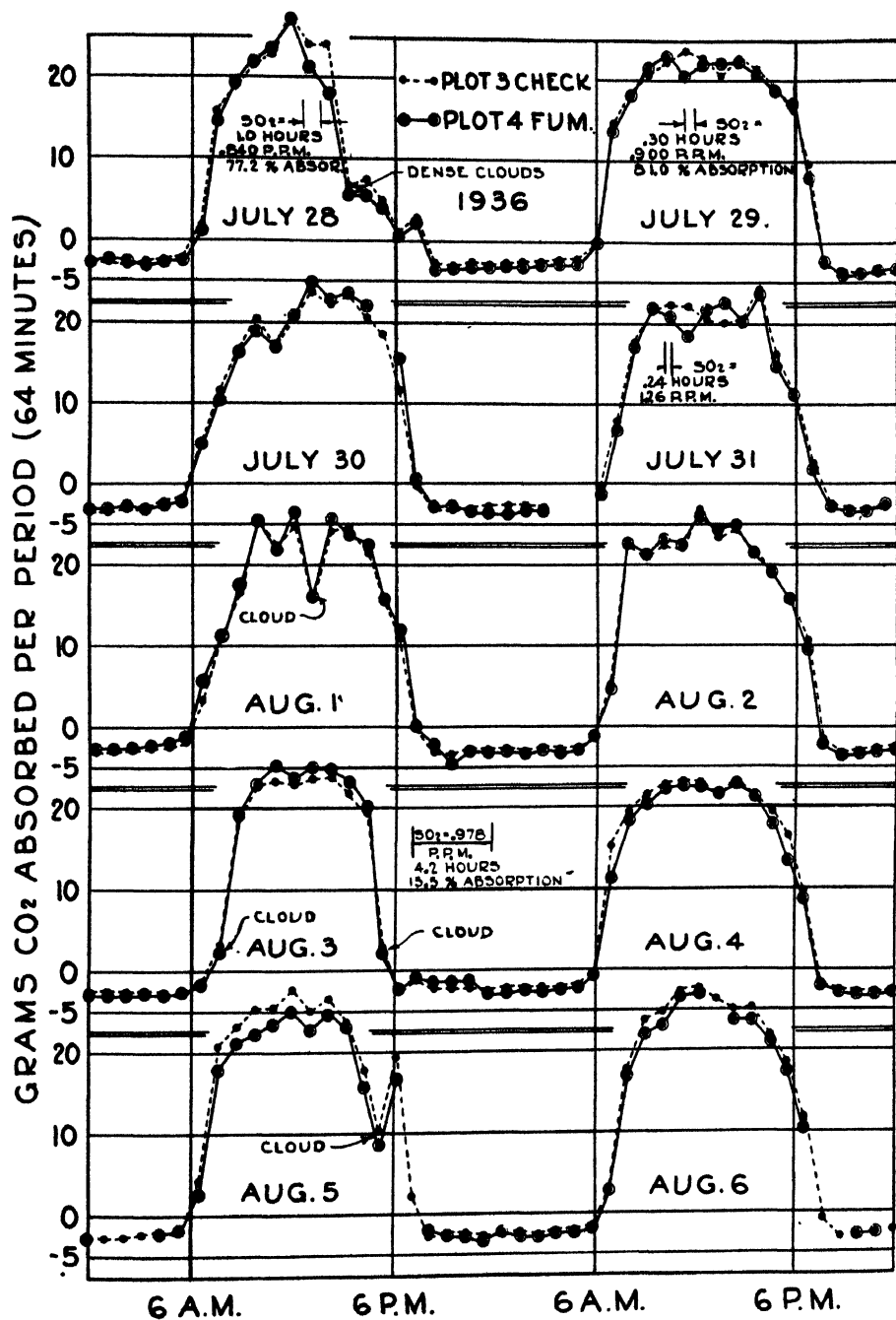


Fig. 3. Chart B, July 28-August 6, 1936.



TABLE IV  
BALANCE SHEET OF CARBON DIOXIDE EXCHANGE AND GROWTH OF ALFALFA  
PLOTS 3 AND 4—1936

DATE (1936)	TOTAL APPARENT ASSIMILATION CO <sub>2</sub>				NIGHT RESPIRATION CO <sub>2</sub>				NET ASSIMILATION CO <sub>2</sub>			
	PLOT 3 CHECK	PLOT 4 FUMI- GATED	RATIO 4/3		PLOT 3 CHECK	PLOT 4 FUMI- GATED	RATIO 4/3		PLOT 3 CHECK	PLOT 4 FUMI- GATED	RATIO 4/3	
July 15-20*	gm.	gm.	%		gm.	gm.	%		gm.	gm.	%	
July 21	926.9	928.4	100	99 <sup>a</sup>	182.8	212.5	116		744.1	715.9		96
" "				82 <sup>b</sup>								
" "				100 <sup>c</sup>								
" "	179.4	165.6	92 <sup>d</sup>		27.2	28.4	104		152.2	137.2		90
July 22*	187.3	184.2	98		29.0	32.4	112		158.3	151.8		96
July 23				111 <sup>a</sup>								
" "				92 <sup>b</sup>								
" "	189.4	189.0	105 <sup>c</sup>		27.7	29.8	108		161.7	159.2		98
" "	203.4	208.4	102		28.9	33.8	117		174.5	174.6		100
July 24*				104 <sup>a</sup>								
July 25				56 <sup>b</sup>								
" "				104 <sup>c</sup>								
" "	216.4	209.4	97 <sup>d</sup>		27.7	26.7	96		188.7	182.7		97
July 26-27*	429.9	449.1	104		52.6	56.0	106		377.3	393.1		104
July 28				100 <sup>a</sup>								
" "				75 <sup>b</sup>								
" "				80 <sup>c</sup>								
" "	179.4	164.6	92 <sup>d</sup>		23.2	28.5	123		156.2	136.1		87
July 29				101 <sup>a</sup>								
" "				87 <sup>b</sup>								
" "				100 <sup>c</sup>								
" "	234.5	229.7	98 <sup>d</sup>		30.8	30.5	99		203.7	199.2		98

**TABLE IV—(Continued)**

to be depressed during the treatment, but became normal soon afterward. Conspicuous acute markings developed the next day. Acute leaf destruction, measured from a small sample on August 5, was 12 per cent. of the total leaf area, and chlorotic markings were 14 per cent. At harvest on August 8, these values were 3 per cent. and 11 per cent., respectively. The rate of apparent assimilation was reduced 8 per cent. on August 4 and 5, and 3 per cent. on August 6 and 7, showing that interference with photosynthesis paralleled leaf destruction, and showing also that recovery was practically complete in 4 days.

Table IV shows that the apparent assimilation values of the two plots, which were equal during the pre-fumigation period from July 15 to July 20, were not significantly different over the whole period of the experiments, although measurable reductions in the rate of apparent assimilation occurred during each of the fumigations. Table IV and figure 3 indicate a tendency for the photosynthetic level of the treated plot to rise above the level of the check plot during part of the afternoon following the treatment, and also on the following day, suggesting a compensating stimulation immediately following the fumigation. This tendency, though slight, is sufficient to offset practically all of the reductions due to the fumigations. The harvest weights of the two crops were not significantly different.

A number of complete shoots were removed from the plots at intervals for chemical analyses. These shoots were taken at random from all parts of the plot through the 5 sample windows with which the fumigation cabinets were provided. All the samples were taken as nearly as possible at the same time of day so that they would be comparable in carbohydrate content. The leaves were immediately separated from the stems. When the plots were finally harvested, equal portions from each plot were cut simultaneously to avoid differences in carbohydrate content of the leaves, and a complete separation of leaves and stems was made. Portions of these samples were analyzed immediately for chlorophyll by the method of WILLSTÄTTER and STOLL, modified by SCHERTZ (3), using GUTHRIE'S (1) ammoniacal copper sulphate-potassium dichromate solution as the working reference. The latter was standardized against preparations from known amounts of pure pigment. Chlorophyll data for leaves and stems are given in table V. They indicate that the fumigated plot remained practically constant in chlorophyll content throughout the period of the experiment, whereas the check plot increased a little in this constituent, while the plants growing outside of the plant chamber decreased slightly in chlorophyll. Evidently the slightly reduced amount of chlorophyll in the treated leaves as compared with the check leaves did not impair measurably their photosynthetic activity. Variations of chlorophyll in the stems are without significance.

TABLE V  
ANALYSES OF PLOTS 3 AND 4 FOR SULPHUR AND CHLOROPHYLL (DRY BASIS)—1936

DATE (1936)	PLANT TISSUE	SULPHUR (S)						CHLOROPHYLL			
		PLOT 3 (CHECK)			PLOT 4 (FUMIGATED)			PLOT 3 CHECK	PLOT 4 FUMI- GATED	OUTSIDE* CHECK	
		WEIGHT OF TISSUE	SULPHUR (S)	WEIGHT (S)	WEIGHT OF TISSUE	SULPHUR (S)	WEIGHT (S)				
July 15	Leaves	gm.	%	gm.	gm.	%	gm.	%	%	1.69	
" 20	"							1.80	1.73		
" 26	"							1.81	1.57	1.60	
" 29	"							2.02	1.60		
August 8	Normal leaves	606	0.503	3.05	395	0.814	3.22	1.97	1.68	1.53	
" 8	Marked "				225	0.762	1.71		1.42		
July 20	Stems							0.21	0.24		
" 26	"							0.22	0.26	0.22	
" 29	"							0.24	0.23		
August 8	"	1570	0.162	2.54	1515	0.197	2.98	0.27	0.29	0.19	
" 8	Dead tissue	120	0.468	0.56	115	0.445	0.51				
" 8	Leaves and stems		0.257			0.371					
Total		6.15						8.42			
Excess of sulphur in plot 4 over plot 3								2.27			

\* Comparable alfalfa grown adjacent to and outside of plant chambers.

TABLE VI

FUMIGATION DATA OF PLOT 12—1936

DATE (1936)	FUMIGATION		AIR VELOCITY	CONCENTRATION OF SO <sub>2</sub>		SO <sub>2</sub> ABSORBED	LEAF DESTRUCTION	
	STARTED	DURATION		INTAKE	OUTLET		ACUTE	CHLOROTIC
		min.	l.p.m.	p.p.m.	p.p.m.	%	%	%
August 8	9:30 A.M.	254	5530	0.579	0.424	26.8	8.1	.....
" 9	9:22 A.M.	254	5520	0.574	0.423	26.3	0.47	.....
" 10	8:59 A.M.	252	5470	0.567	0.412	27.2	0.47	1.0
Av. Aug. 8-10	.....	253	5510	0.573	0.420	26.8	0.473	.....
August 15	8:56 A.M.	240	5500	0.603	0.460	23.7	0.42	.....
" 16	8:59 A.M.	240	5390	0.622	0.452	27.3	0.49	3.0
" 17	8:57 A.M.	240	5470	0.657	0.382	41.9	0.80	.....
Av. Aug. 15-17	.....	240	5450	0.627	0.431	31.0	0.57	2.3
August 21 <sup>a</sup>	.....	.....	.....	.....	.....	.....	.....	.....
Duration of fumigations (hours)						24.7		
Total weight of SO <sub>2</sub> absorbed (grams)						3.13		
Total weight of S absorbed (grams)						1.57		

\* Milligrams SO<sub>2</sub> absorbed per minute per 1000 gm. dry leaves.<sup>a</sup> 0.9 per cent. weather discolorations on check (plot 11).

Analyses of the leaves and the stems for total sulphur at harvest are also given in table V. It will be noted that the sulphur content of the fumigated leaves was about 0.8 per cent. as compared with 0.5 per cent. in the check leaves. The fumigated stems were also somewhat higher in sulphur than the check stems. When the total amount of sulphur in the system was calculated, it was found that there was an excess of 2.27 gm. of sulphur in plot 4 over plot 3. This is appreciably greater than the total weight of sulphur absorbed, 1.43 gm., as indicated by the gas analyses in table III. This discrepancy can be ascribed to the fact that plot 4 had been given an extensive fumigation in 1935, described below, which had enriched the roots and permitted additional sulphur nutrition on subsequent crops. This point will be referred to again.

D. EFFECT OF FUMIGATIONS WITH APPROXIMATELY 0.6 P.P.M.—Plots 11 and 12 of third-crop alfalfa were observed from August 5 to August 20, 1936. This variety of alfalfa is Province no. 34886. The alfalfa had been previously cut on June 26 and July 27. The fumigation data of plot 12 are presented in table VI. Plot 12 received 3 fumigations, each of 4.2 hours duration, on the mornings of August 8, 9, and 10. The average intake concentration was 0.573 p.p.m., the outlet 0.420 p.p.m., and the mean 0.497 p.p.m. Then following a rest period of 4 days, plot 12 was again fumigated on 3 successive mornings with 3 fumigations, each of 4.0 hours duration, with an intake concentration of 0.627 p.p.m., the outlet 0.433 p.p.m., and the mean 0.530 p.p.m. Both acute and chlorotic markings could be seen scattered generally over the plot. When the actual leaf areas destroyed were measured at harvest time, the amounts were 2.5 per cent. One small plant, stunted by bacterial wilt, had 50 per cent. acute markings.

Curves of photosynthetic activity and respiration of plots 11 and 12 are presented in figure 4, charts A and B, and the carbon dioxide exchange data are summarized and compared in table VII. The treatments in every case reduced the photosynthetic activity of the treated plot during the time that the fumigation was in progress, but within 2 or 3 hours after the fumigation was discontinued the plot was practically normal again in its activity. The average rate of assimilation on plot 12 during the progress of the first 3 fumigations was 76 per cent. of the check, and 74 per cent. during the second 3 fumigations. Considering the 4 hours of the afternoons on these days, starting in each case about an hour after the fumigation had been discontinued, the average rate of photosynthesis on the treated plot was 98 per cent. of the check in the first 3 fumigations, and 88 per cent. in the second 3 fumigations. During the 4-day rest period between the two series of fumigations, the rate of photosynthesis of plot 12 was 98 per cent. of plot 11, and during 3 days following the last treatment, 93 per cent. of the check.

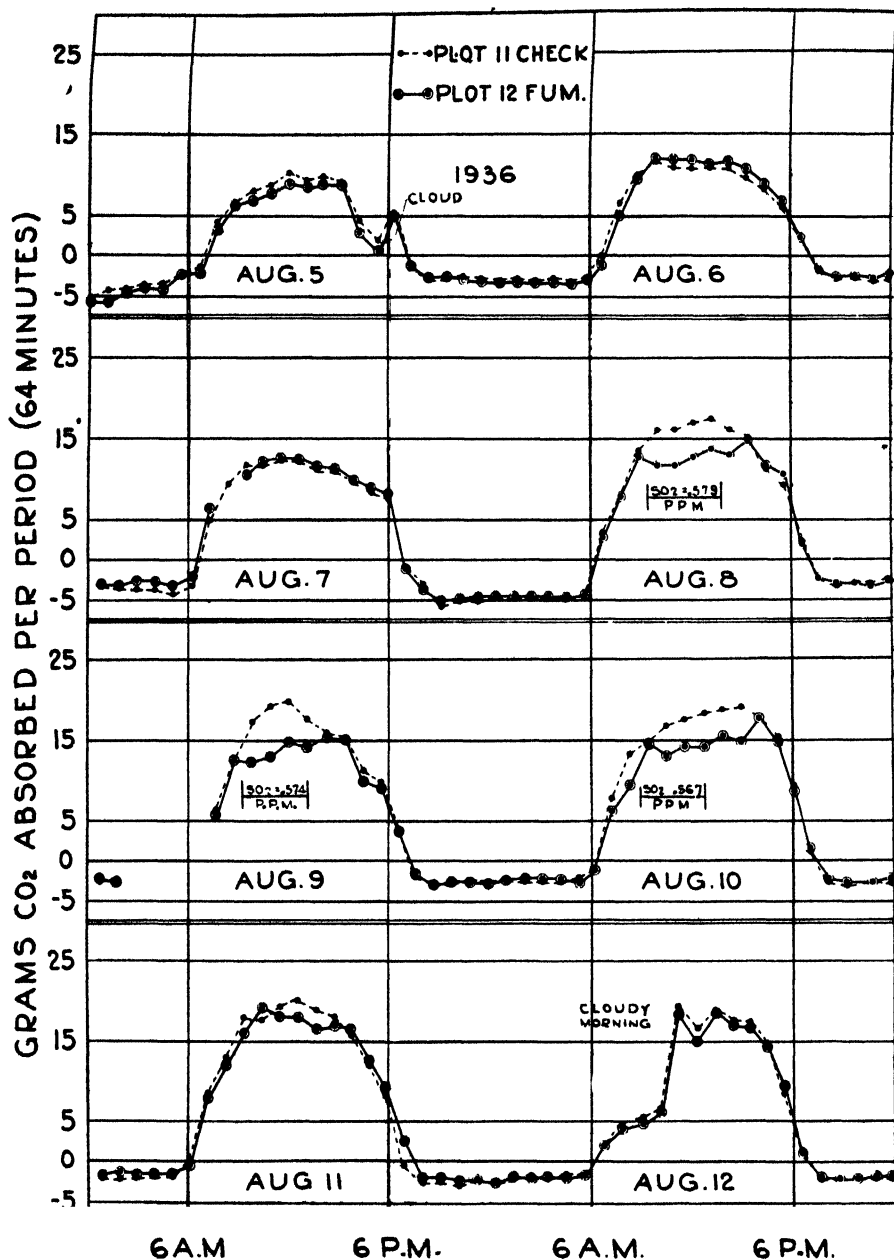


FIG. 4. Chart A, August 5-12, 1936. Apparent assimilation and respiration of plots 11 and 12 at Logan, Utah. Plot 12 received six 4-hour fumigations with about 0.6 p.p.m. SO<sub>2</sub> on six mornings. The curves show that photosynthesis on plot 12 is depressed during the treatment, but a rapid recovery occurs when the treatment is discontinued.

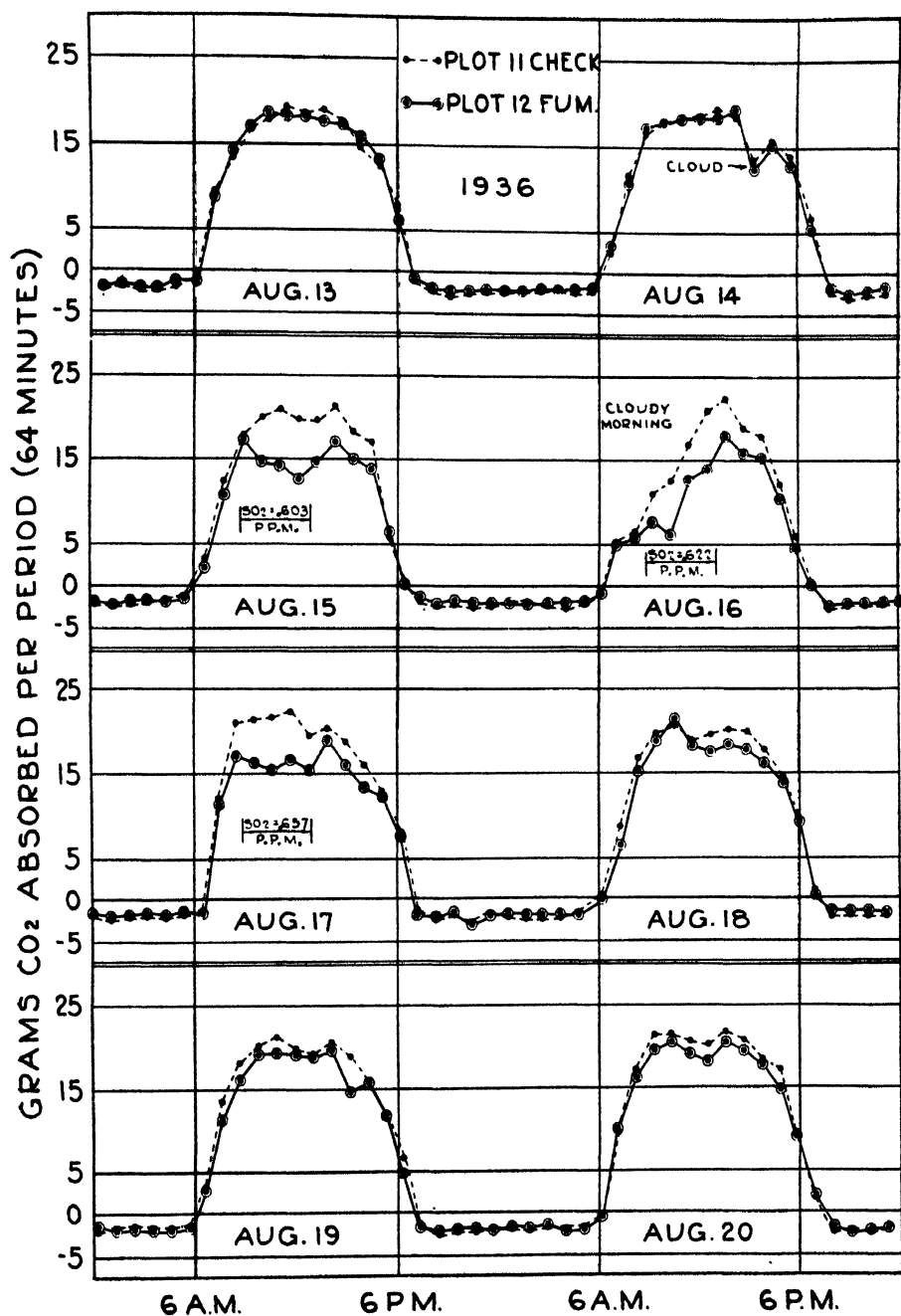


FIG. 4. Chart B, August 13-20, 1936.



TABLE VII  
BALANCE SHEET OF CARBON DIOXIDE EXCHANGE AND GROWTH OF ALFALFA  
PLOTS 11 AND 12—1936

DATE (1936)	TOTAL APPARENT ASSIMILATION CO <sub>2</sub>			NIGHT RESPIRATION CO <sub>2</sub>			NET ASSIMILATION CO <sub>2</sub>		
	PLOT 11 CHECK	PLOT 12 FUMIGATED	RATIO 12/11	PLOT 11 CHECK	PLOT 12 FUMIGATED	RATIO 12/11	PLOT 11 CHECK	PLOT 12 FUMIGATED	RATIO 12/11
August 5-7	gm. 279.3	gm. 277.4	% 99	gm. 91.7	gm. 88.3	% 96	gm. 187.6	gm. 189.1	% 101
August 8-10			95 <sup>a</sup>						
“			76 <sup>b</sup>						
“			98 <sup>c</sup>						
“	472.1	403.9	86 <sup>d</sup>	73.7	70.5	96	398.4	333.4	84
August 11-14	642.7	627.8	98	92.7	81.2	88	550.0	546.6	99
August 15-17			92 <sup>a</sup>						
“			74 <sup>b</sup>						
“			88 <sup>c</sup>						
“	524.1	417.9	80 <sup>d</sup>	65.7	59.0	90	458.4	358.9	78
August 18-20	576.0	533.2	93	52.2	47.5	91	523.8	485.7	93
Totals	2494	2260	91	376	347	92	2118	1914	90
Equivalent dry matter (grams) (44 per cent. carbon)									
Dry matter August 21 (grams at harvest)				1311	1187		1311	1187	
Dry matter August 5 (estimated from weight of equivalent plot harvested August 5) (grams)				1123	966		1123	966	86%
Top growth during experiment (grams)				340	320		340	320	
Top growth—percentage of total CO <sub>2</sub> assimilated				783	646		783	646	
Probable root increment (grams)				58.9%	54.5%		58.9%	54.5%	
				528	541		528	541	

<sup>a</sup> Before fumigation.    <sup>b</sup> During fumigation.    <sup>c</sup> After fumigation.    <sup>d</sup> Total for the period.

TABLE VIII  
ANALYSES OF PLOTS 11 AND 12 FOR SULPHUR AND CHLOROPHYLL (DRY BASIS)—1936

DATE (1936)	PLANT TISSUE	SULPHUR (S)						CHLOROPHYLL			
		PLOT 11 (CHECK)			PLOT 12 (FUMIGATED)			PLOT 11 CHECK	PLOT 12 FUMI- GATED	OUTSIDE* CHECK	
		WEIGHT OF TISSUE	SULPHUR (S)	WEIGHT (S)	WEIGHT OF TISSUE	SULPHUR (S)	WEIGHT (S)				
		gm.	%	gm.	gm.	%	gm.				
August 5	Leaves									%	%
" 9	"									1.25	1.54
" 12	"									1.50	1.47
" 14	"									1.47	1.71
" 17	"	11.3	0.542	0.061	9.1	0.851	0.077	1.76	1.63	1.63	1.71
" 22	Upper leaves	19.8	0.585	0.116	15.3	1.13	0.173	1.62	1.53	1.53	1.42
" 22	Lower "	315.0	0.600	1.890	264.0	1.050	2.772	1.73	1.63	1.63	1.62
" 22	Stems	71.0	0.685	0.486	75.0	1.273	0.955	1.86	1.66	1.66	0.35
August 5	"										0.29
" 9	"							0.38	0.32	0.32	0.30
" 12	"							0.35	0.36	0.36	
" 14	"	15.3	0.226	0.035	11.2	0.265	0.030	0.35	0.36	0.36	
" 17	"	26.2	0.207	0.054	21.6	0.275	0.059	0.36	0.33	0.33	
" 22	"	615.0	0.199	1.222	538.0	0.225	1.210	0.34	0.34	0.34	0.33
" 22	Dead tissue	34.0	0.450	0.153	22.0	0.621	0.137				
" 22	Leaves and stems		0.360			0.563					
Total		4.017									5.413
Excess of sulphur in plot 12 over plot 11											1.396

\* Comparable alfalfa grown adjacent to and outside of plant chambers.

The reduction in the amount of net assimilation which can be ascribed to the first 3 treatments was 3 per cent. of the total net assimilation. The last 3 treatments increased this reduction to 10 per cent. The period of observation in these experiments was only 16 days. As the normal growth period of a crop of alfalfa is at least 30 to 36 days, these percentages should be reduced to about 1.5 and 5 per cent. respectively. The harvest weight of plot 12 was 86 per cent. of the yield of plot 11. This reduced yield was due in part to the fumigation treatments and in part to the fact that the density of the stand on the treated plot was less than on the check. This was indicated by the appearance of the plots, since plot 12 had a somewhat less complete cover than plot 11, and also by the fact that there were 5 per cent. fewer average sized stems on plot 12 than on plot 11.

Chlorophyll and sulphur determinations on plots 11 and 12, similar to those described above for plots 3 and 4, are given in table VIII. Again, the check plot increased in chlorophyll content during the progress of the experiment a little more rapidly than the treated plot, but the differences are without practical significance.

The sulphur analyses of the leaves and stems show an increment of 1.39 gm. of sulphur in plot 12, which may be ascribed to the fumigation. This may be compared with a value of 1.57 gm. given by the air analyses in table VI. As the air analyses were not corrected for absorption by the fumigation cabinet or the soil, the agreement between the two values is quite satisfactory. Neither of these plots had been fumigated previously.

E. EFFECT OF ABOUT 0.4 P.P.M.—Plots 9 and 10 were investigated from August 8 to 31, 1936. This variety of alfalfa is Turkestan no. 19315. The fumigation data for plot 9 are given in table IX. Plot 9 received 5 fumigations, each of 4.2 hours duration, on 5 successive mornings, beginning between 8 and 10 A.M., the average concentrations being 0.417 p.p.m. intake, 0.231 p.p.m. outlet, and 0.324 p.p.m. mean. Plot 10 was a check. Then following a rest period of 5 days, plot 9 was given another series of 5 fumigations each of 4.2 hours duration on 5 successive mornings, the average intake concentration being 0.441 p.p.m., the average outlet concentration 0.237 p.p.m., and the mean 0.339 p.p.m. These treatments possibly produced some slight acute markings and also some chlorotic markings; but at harvest time it was difficult to distinguish markings due to sulphur dioxide, and those due to other causes. There were 0.6 per cent. more discolorations on the fumigated plot than on the check plot.

Carbon dioxide exchange data for plots 9 and 10 are given in figure 5, charts A and B, and are summarized in table X. The reduction in the rate of apparent assimilation during the time each fumigation was in progress averaged about 9 per cent. during the first 5 treatments and about 14 per cent. during the second 5 treatments. It is interesting to note that the net assimilation of plot 9 was 97 per cent. of the check plot for the 3 days

TABLE IX  
FUMIGATION DATA OF PLOT 9—1936

DATE (1936)	FUMIGATION		AIR VELOCITY	CONCENTRATION OF SO <sub>2</sub>		SO <sub>2</sub> ABSORBED		LEAF DESTRUCTION	
	STARTED	DURA- TION		INTAKE	OUTLET			ACUTE	CHLOROTIC
		min.	<i>l.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>	%	<i>gm.</i>	%	%
August 13	10: 03 A.M.	251	5040	0.427	0.202	52.6	0.634	9.4	.....
" 14	8: 41 A.M.	251	5850	0.388	0.217	44.0	0.555	7.7	.....
" 15	8: 44 A.M.	252	5730	0.421	0.225	46.5	0.625	8.5	.....
" 16	8: 57 A.M.	252	5770	0.430	0.265	38.3	0.534	6.8	.....
" 17	8: 40 A.M.	251	5820	0.417	0.243	41.8	0.566	6.8	Traces
Av. Aug. 13-17	.....	251	5642	0.417	0.231	44.6	0.583	.....	.....
August 23 <sup>a</sup>	9: 03 A.M.	253	5500	0.440	0.239	45.7	0.619	1.1	2.6
" 24	8: 44 A.M.	254	5420	0.440	0.235	46.7	0.628	.....	.....
" 25	9: 04 A.M.	253	5440	0.456	0.231	49.4	0.691	.....	.....
" 26 <sup>b</sup>	8: 53 A.M.	254	5450	0.440	0.240	45.5	0.618	1.0	1.6
" 27	8: 50 A.M.	253	5475	0.429	0.239	44.3	0.593	.....	.....
Av. Aug. 23-27	.....	253	5457	0.441	0.237	46.3	0.630	.....	.....
September 2 <sup>c</sup>	.....	.....	.....	.....	.....	.....	.....	0.2	1.2
Duration of fumigations (hours) .....						42.1			
Total weight of SO <sub>2</sub> absorbed (grams) .....						6.05			
Total weight of S absorbed (grams) .....						3.02			

\* Milligrams SO<sub>2</sub> absorbed per minute per 1000 gm. dry leaves.

<sup>a</sup> 1.5 per cent. weather discolorations on check (plot 10).

<sup>b</sup> 1.5 per cent. weather discolorations on check (plot 10).

<sup>c</sup> 0.8 per cent. weather discolorations on check (plot 10).

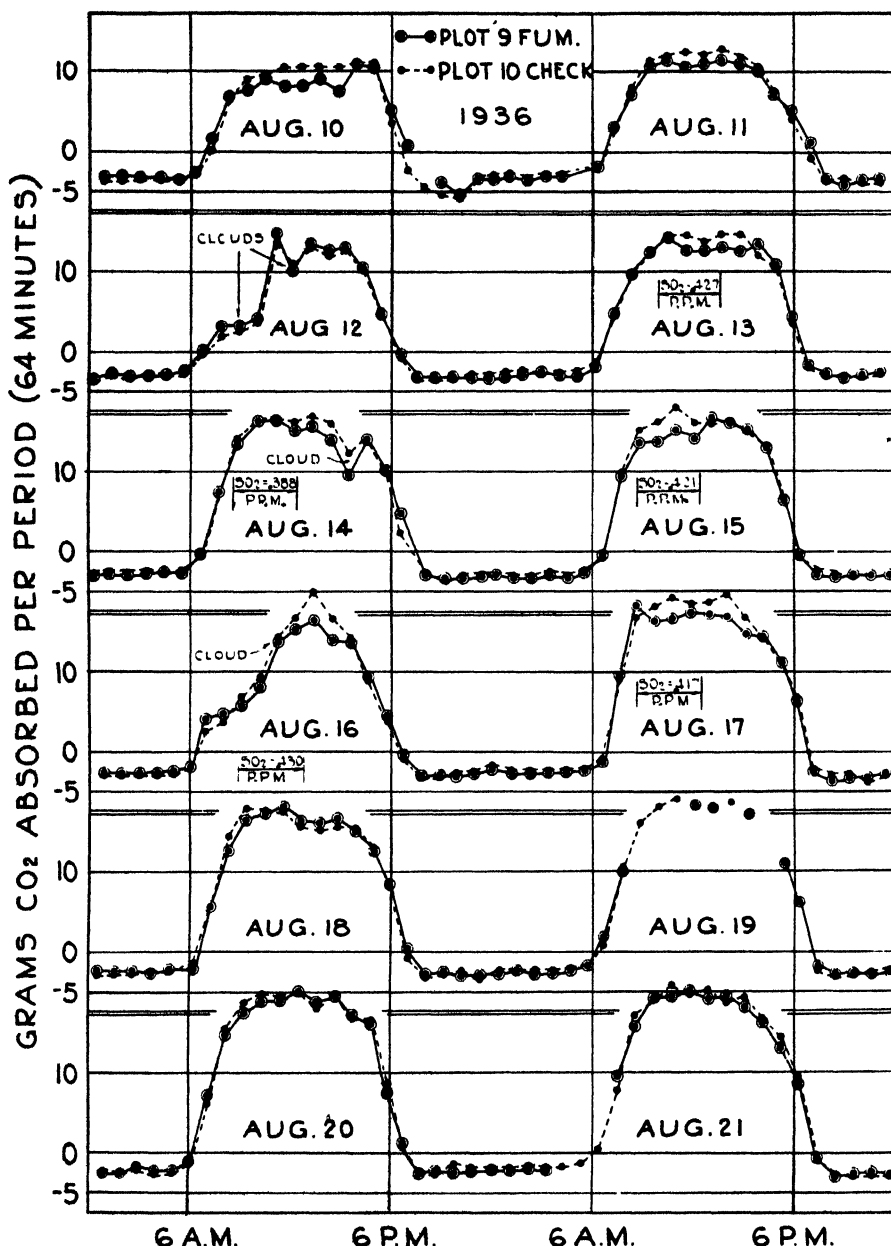


FIG. 5. Chart A, August 10-21, 1936. Apparent assimilation and respiration of plots 9 and 10 at Logan, Utah. The effects of ten 4-hour fumigations on plot 9 with 0.44 p.p.m. SO<sub>2</sub> on ten mornings are shown.

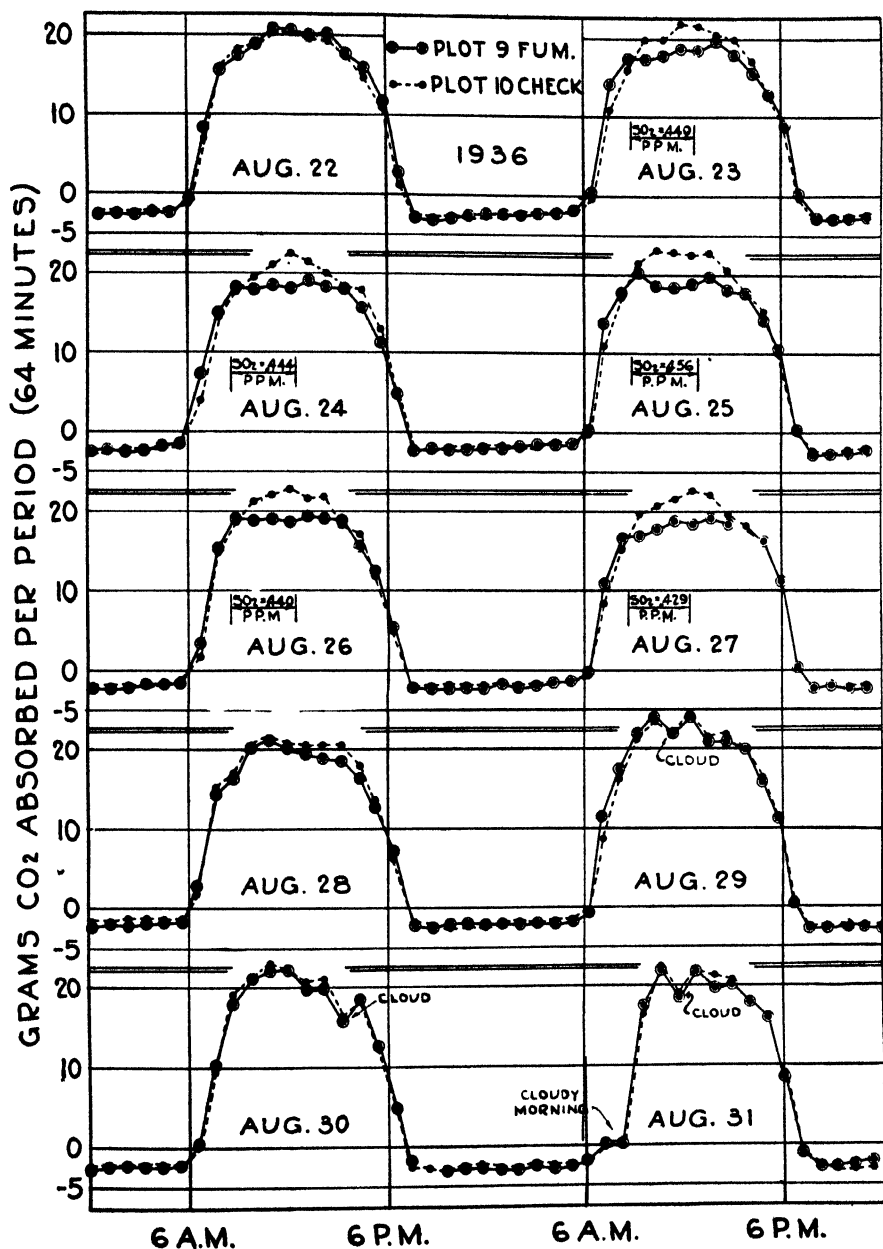


FIG. 5. Chart B, August 22-31, 1936.

TABLE X

BALANCE SHEET OF CARBON DIOXIDE EXCHANGE AND GROWTH OF ALFALFA PLOTS 9 AND 10—1936

DATE (1936)	TOTAL APPARENT ASSIMILATION CO <sub>2</sub>			NIGHT RESPIRATION CO <sub>2</sub>			NET ASSIMILATION CO <sub>2</sub>		
	PLOT 10 CHECK	PLOT 9 FUMI- GATED	RATIO 9/10	PLOT 10 CHECK	PLOT 9 FUMI- GATED	RATIO 9/10	PLOT 10 CHECK	PLOT 9 FUMI- GATED	RATIO 9/10
August 10-12	gm. 288.7	gm. 282.0	% 98	gm. 98.9	gm. 97.2	% 98	gm. 189.8	gm. 184.8	% 97
August 13-17	.....	.....	102 <sup>a</sup>	.....	.....	.....	.....	.....	.....
"	.....	.....	91 <sup>b</sup>	.....	.....	.....	.....	.....	.....
"	.....	.....	98 <sup>c</sup>	.....	.....	.....	.....	.....	.....
"	697.0	661.7	95 <sup>d</sup>	145.6	152.3	105	551.4	509.4	92
August 18-22	871.0	878.2	101	122.0	121.5	100	749.0	756.7	101
August 23-27	.....	.....	105 <sup>a</sup>	.....	.....	.....	.....	.....	.....
"	.....	.....	86 <sup>b</sup>	.....	.....	.....	.....	.....	.....
"	.....	.....	96 <sup>c</sup>	.....	.....	.....	.....	.....	.....
"	983.0	920.7	94 <sup>d</sup>	98.6	104.3	106	884.4	816.4	92
August 28-31	759.5	750.2	99	98.8	97.0	98	660.7	653.2	99
Totals	3599	3493	97.0	564	572	101.5	3035	2920	96.2
Equivalent dry matter (grams) (44 per cent. carbon)									
Dry matter September 1 (grams at harvest) ..... 1882 1810									
Dry matter August 10 (estimated from weight of equivalent plot harvested August 10) (grams) ..... 1424 1443									
Top growth during experiment (grams) ..... 485 478									
Top growth—percentage of total CO <sub>2</sub> assimilated ..... 939 965									
Probable root increment (grams) ..... 49.7 53.3									
..... 943 945									

immediately preceding the first 5 fumigations. It was 101 per cent. of the check on the 5 days succeeding these fumigations, and 99 per cent. of the check in the period following the second 5 fumigations, again suggesting a stimulation of photosynthesis by sulphur dioxide at this exposure and dilution. These increases in net assimilation largely counterbalance the reductions due to the fumigations, so that the total net assimilation of the treated plot for the period of the experiment was 96 per cent. of the check as compared with 97 per cent. in the period before fumigation treatments were started. This difference between the net assimilation values of the 2 plots is probably without significance. The 1 per cent. difference between the actual yields of the plots at harvest time in favor of the fumigated plot certainly is without significance.

Chlorophyll analyses on plots 9 and 10, given in table XI confirm the conclusions stated for the preceding plots, that there was no effect of practical significance of these fumigations on the chlorophyll.

The sulphur analyses in table XI show that there was an excess of sulphur in plot 9 over plot 10 of 2.73 gm., which can be attributed entirely to the fumigation treatments, since neither of these plots had been fumigated previously. This value compares favorably with the absorption value of 3.02 gm. determined by air analyses, as indicated in table IX. The sulphur content of fumigated leaves was increased by these fumigations to double the content of the check leaves.

F. EFFECT OF A SERIES OF INTERMITTENT FUMIGATIONS OF ABOUT 0.3 P.P.M.—Plots 5 and 6 were under observation from August 24 to October 4, 1936. The variety of alfalfa was Turkestan no. 19303. This is the same pair of plots that was used in the second experiment in 1935, described later, and also in an earlier experiment in 1936. In each case, plot 6 was fumigated and plot 5 was the check. The first crop had been harvested on June 26, and the second crop on August 3. The fumigation data for plot 6 are given in table XII. Plot 6 was fumigated each morning from August 30 to September 30, inclusive, except September 1 when it was not fumigated, and except for 4 other days when it was fumigated in the afternoon instead of in the morning. The plot thus received 31 fumigations, each of 4.25 hours, totaling 132 hours. The average intake concentration was 0.348 p.p.m., the average outlet concentration 0.228 p.p.m., and the mean 0.288 p.p.m. In this experiment, in addition to observing the effect of the fumigation treatment, the plots were purposely allowed to go without irrigation water for 3 weeks in order to observe whether the addition of an unfavorable factor, such as drought, would have any effect upon the fumigation treatments. During the hot part of 3 days—September 17, 18, and 19—both plots wilted perceptibly, the check plot suffering somewhat more at this time than the treated plot. Thereafter they were both watered adequately. As



TABLE XI  
ANALYSES OF PLOTS 9 AND 10 FOR SULPHUR AND CHLOROPHYLL (DRY BASIS)—1936

DATE (1936)	PLANT TISSUE	SULPHUR (S)				CHLOROPHYLL			
		PLOT 10 (CHECK)		PLOT 9 (FUMIGATED)		PLOT 10 CHECK	PLOT 9 FUMI- GATED	OUTSIDE* CHECK	
		WEIGHT OF TISSUE	SULPHUR (S)	WEIGHT (S)	WEIGHT OF TISSUE	SULPHUR (S)	WEIGHT (S)		
		gm.	%	gm.	gm.	%	gm.	%	%
August 9	Leaves	.....	.....	.....	.....	.....	.....	.....	1.30
" 12	"	.....	.....	.....	.....	.....	.....	.....	1.45
" 14	Upper leaves	8.2	0.512	0.042	8.6	0.795	0.068	1.82	1.46
" 14	Lower "	3.1	0.546	0.017	2.4	0.728	0.017	1.87	.....
" 17	Upper "	9.4	0.582	0.055	13.5	0.923	0.125	1.92	.....
" 17	Lower "	.....	.....	.....	1.6	0.805	0.013	1.73	.....
" 23	Upper "	19.1	0.585	0.112	19.6	0.894	0.175	1.79	1.69
" 23	Lower "	3.2	0.575	0.018	1.2	0.863	0.010	2.04	.....
" 26	Upper "	17.1	0.553	0.095	22.5	1.111	0.250	1.82	1.53
" 26	Lower "	5.5	0.601	0.033	7.8	1.068	0.083	2.15	1.76
Sept. 1	Upper "	377.0	0.518	1.953	379.0	1.013	3.840	1.75	1.52
" 1	Lower "	88.0	0.575	0.495	69.0	1.035	0.715	1.75	1.75
Aug. 9	Stems	.....	.....	.....	.....	.....	.....	.....	0.37
" 12	"	.....	.....	.....	.....	.....	.....	.....	0.30
" 14	"	15.6	0.239	0.037	16.2	0.264	0.043	0.28	.....
" 17	"	13.8	0.263	0.036	23.5	0.262	0.062	0.35	.....
" 23	"	39.8	0.186	0.074	34.3	0.221	0.076	0.32	.....
" 26	"	38.0	0.221	0.084	52.0	0.237	0.123	0.29	0.31
Sept. 1	"	750.0	0.207	1.553	736.0	0.221	1.627	0.30	.....
" 1	Dead tissue	32.0	0.440	0.141	51.0	0.478	0.244	0.28	0.26
" 1	Leaves and stems	.....	0.330	.....	.....	0.521	.....	.....	.....
Total		.....	.....	4.745	.....	.....	7.471	.....	.....
Excess of sulphur in plot 9 over plot 10		.....	.....	.....	.....	.....	2.73	.....	.....

\*Comparable alfalfa grown adjacent to and outside of plant chambers.

TABLE XII  
FUMIGATION DATA OF PLOT 6—1936

DATE (1936)	NUMBER OF FUMI- GATIONS	AIR VELOCITY	DURATION EACH FUMI- GATION	SO <sub>2</sub> CONCENTRATION EACH FUMIGATION		SO <sub>2</sub> ABSORBED EACH FUMIGATION			TOTAL S
				INTAKE	OUTLET	%	gm.	rate <sup>a</sup>	
August 30 to September 4	5	<i>l.p.m.</i>	<i>min.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>				<i>gm.</i>
		Maximum <sup>a</sup> 5750	260	0.397	0.306	41.1	0.445	3.1	
		Minimum <sup>a</sup> 5350	252	0.335	0.197	22.4	0.289	2.0	
		Average 5660	255	0.366	0.245	33.2	0.388	2.7	0.86 <sup>b</sup>
September 5 to September 30	26	Maximum <sup>a</sup> 5660	264	0.368	0.260	45.1	0.450	3.0	
		Minimum <sup>a</sup> 5240	253	0.308	0.172	26.7	0.282	1.9	
		Average 5520	255	0.344	0.224	34.9	0.380	2.6	5.05 <sup>b</sup>
Totals	31		7913						5.91

\* Milligrams SO<sub>2</sub> absorbed per minute per 1000 gm. dry leaves.

<sup>a</sup> The maximum and minimum values in the horizontal lines of this table occurred in different fumigations and are, therefore, unrelated.

<sup>b</sup> Absorption of September 4 distributed equally between these totals.

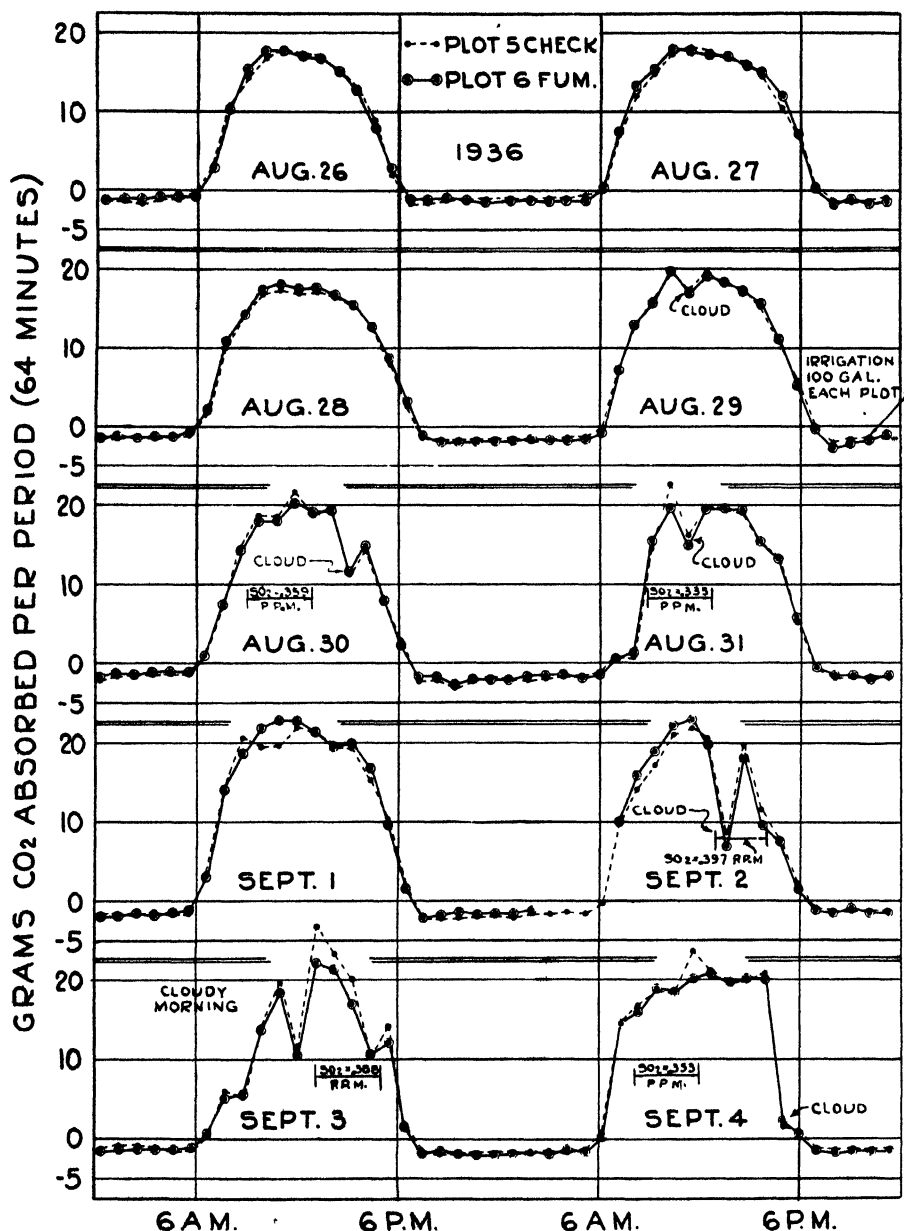


FIG. 6. Chart A, August 26–September 4, 1936. Apparent assimilation and respiration of plots 5 and 6 at Logan, Utah. Plot 6 received 31 4-hour fumigations on 27 mornings and 4 afternoons, with about 0.35 p.p.m. SO<sub>2</sub>. In addition, water was purposely withheld from both plots from August 29 until September 19. Some temporary wilting occurred on September 17, 18, and 19.

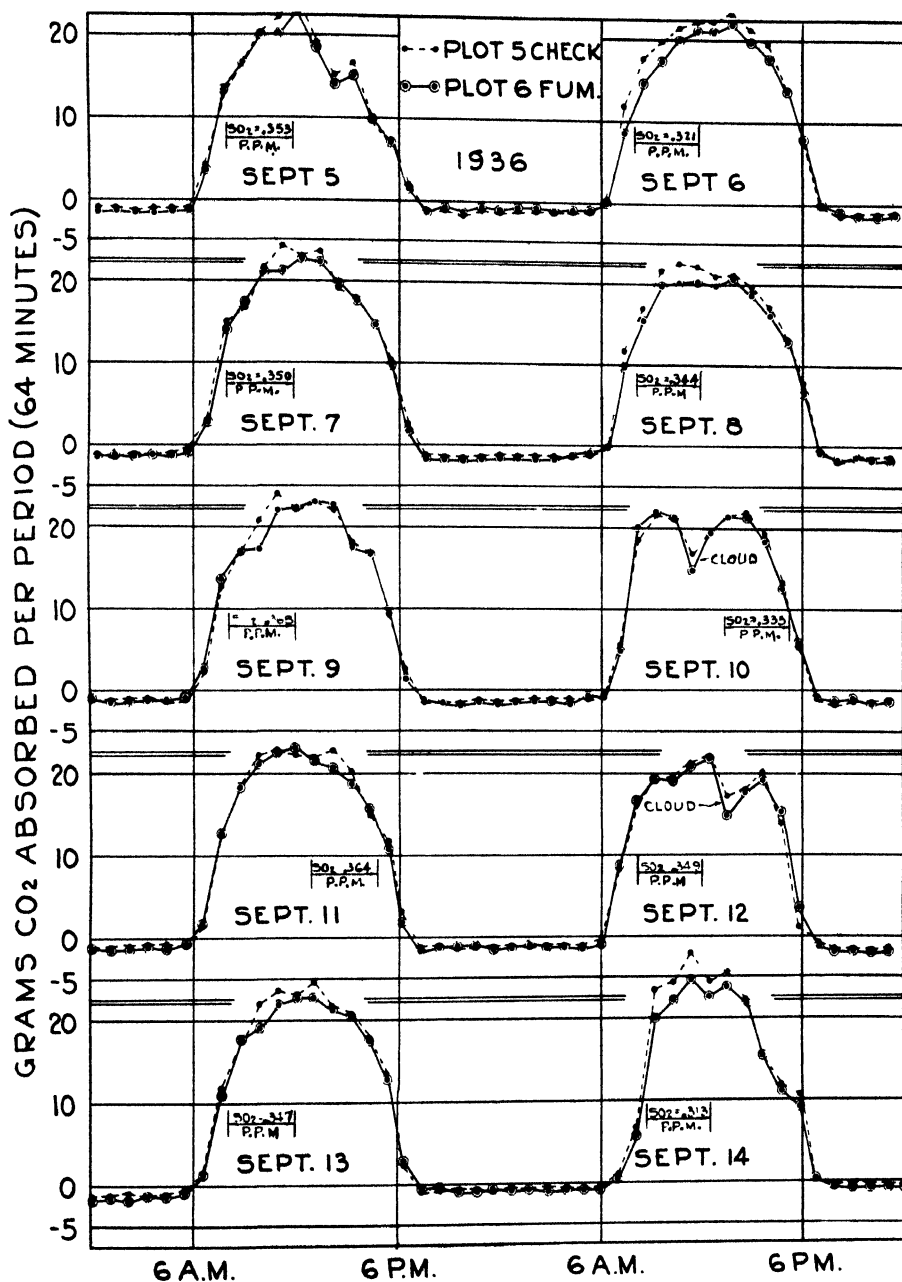


Fig. 6. Chart B, September 5-14, 1936.

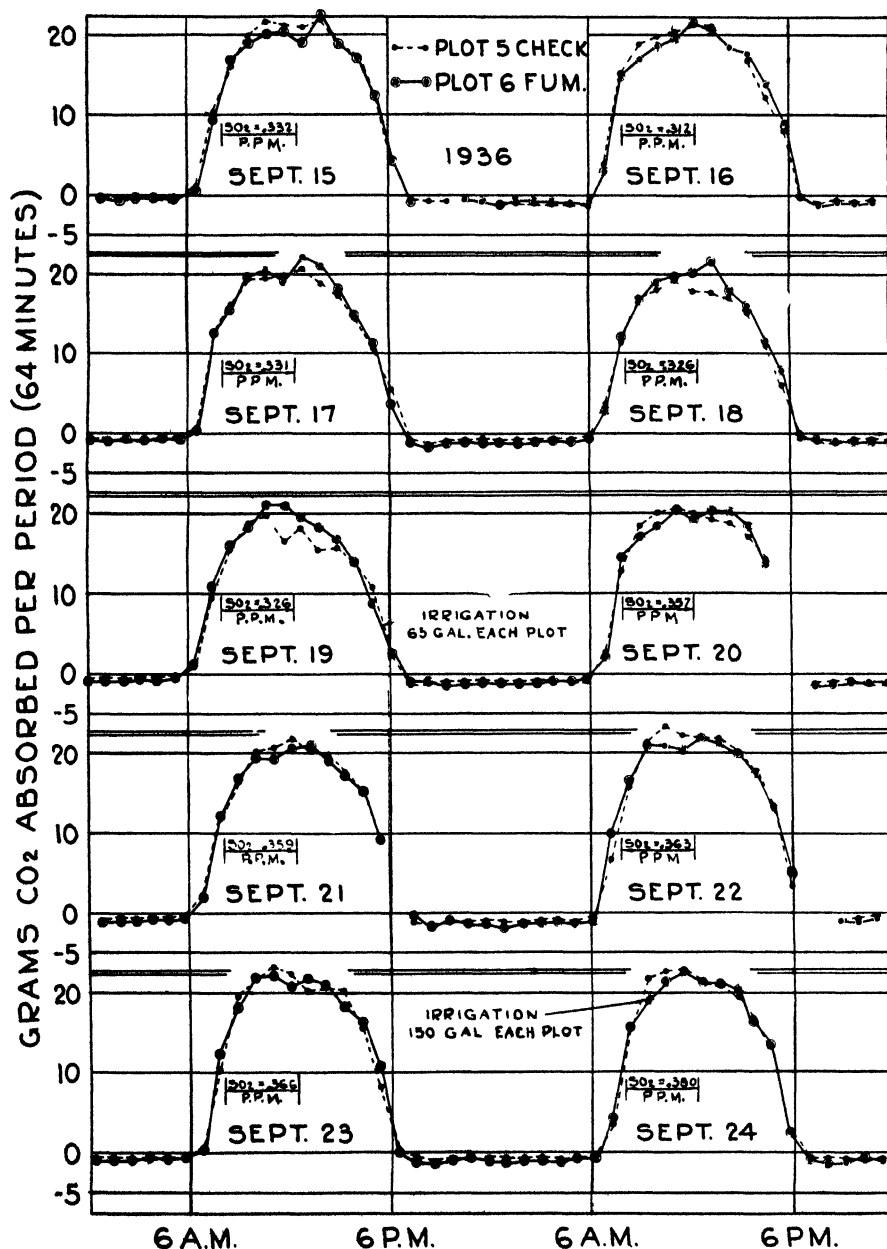


FIG. 6. Chart C, September 15-24, 1936.

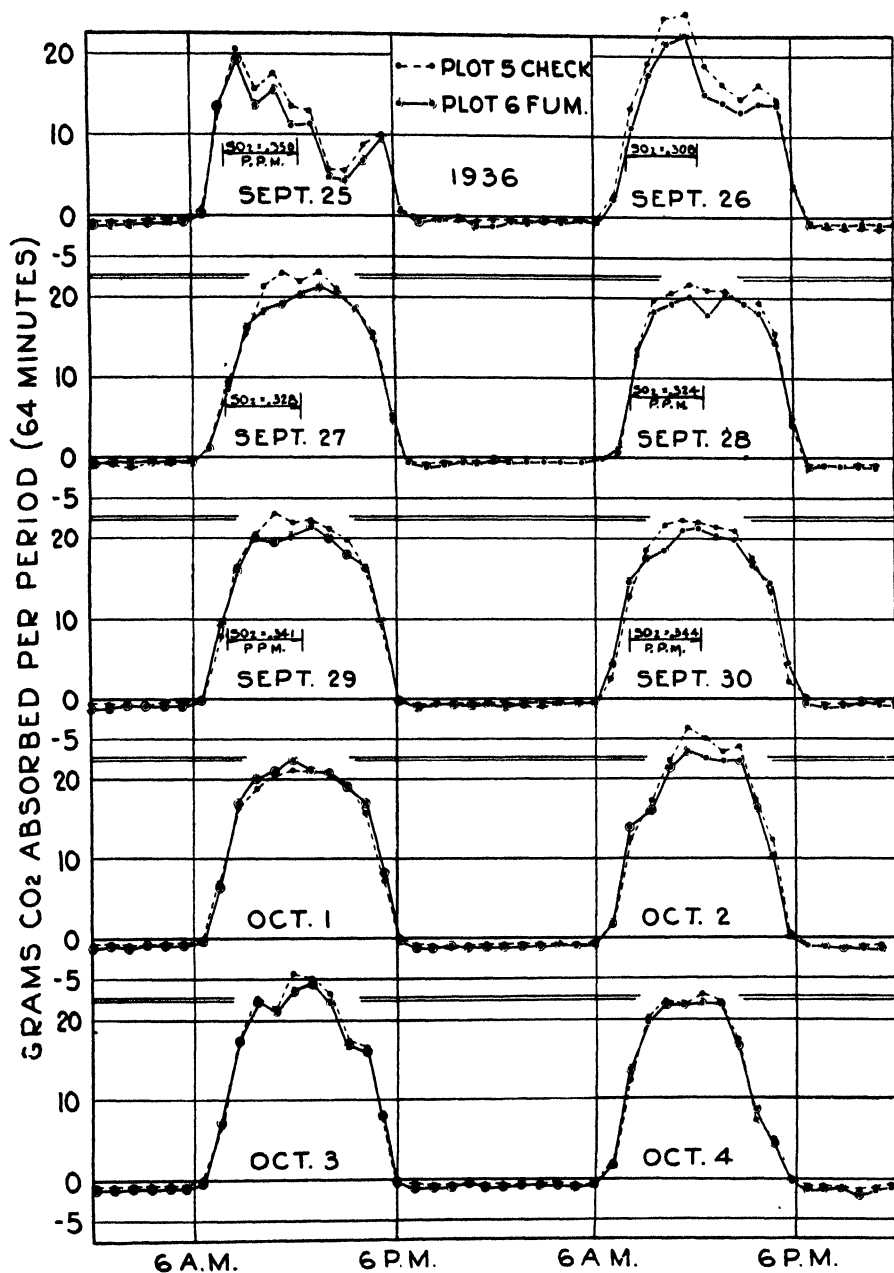


Fig. 6. Chart D, September 25-October 4, 1936.

a result of the drought, and some cold nights (minimum temperature 34° F.), a considerable amount of leaf discoloration and destruction appeared on both plots. Table XIII gives the observed leaf destruction. It will be

TABLE XIII  
LEAF AREA ON PLOTS 5 AND 6 SHOWING DISCOLORATIONS FROM VARIOUS CAUSES

DATE (1936)	PLOT 5 (CHECK)			PLOT 6 (FUMIGATED)		
	BLEACHED	CHLOROTIC	TOTAL	BLEACHED	CHLOROTIC	TOTAL
	%	%	%	%	%	%
September 8		5.7	5.7	0.6	5.9	6.5
“ 19		3.5	3.5	0.3	2.5	2.8
“ 28	2.4	4.8	7.2	0.9	5.5	6.4
October 5	1.0	2.5	3.5	1.7	3.8	5.5

seen that the two plots had nearly the same amounts of lesions. The markings in the fumigation cabinet could not be distinguished from those in the check. Nevertheless, the final measurements, which were the most reliable, show slightly higher values on the treated plot, and the differences could be attributed to the fumigations.

The curves of carbon dioxide exchange are given in figure 6, charts A, B, C, and D, and the data are summarized in table XIV. The curves in figure 6 and table XIV show that from August 30 to September 16, and from September 20 to September 30, apparent assimilation and net assimilation were normal at all times, except the hours of actual fumigation, since the ratios of fumigated to check plots after the fumigations were not significantly different from the ratios before fumigation began. During the time that the fumigation was actually in progress, the rate of assimilation was reduced about 4 per cent. on August 30 and 31, and about 6 per cent. between September 2 and 16. This is from one-third to one-fifth of the amount that the assimilation of both plots was reduced by passing clouds on August 29, 30, 31, September 2, and September 3. During the remainder of each of these days the average assimilation, omitting the hour immediately following the fumigation, was 99 per cent. of the check, which was not a significant reduction. From September 17 until September 19 the treated plot was about 4 per cent. more active than the check in the morning while the sulphur dioxide was present; and during the afternoon when the fumigation was discontinued, it was 7 per cent. more active than the check plot. This was possibly due to the fact that the check plot seemed to be suffering a little more from drought than the treated plot. It is interesting to note that relations between the fumigation and post-fumigation periods were relatively the same on these days as when the plots were adequately watered. On the afternoon of September 19, the plots were irrigated and

on subsequent days the effect of the fumigation again became perceptible, the reduction being about 7 per cent. in the mornings but only 1 per cent. in the afternoons from September 20 to 30. During the final 4-day observation period, after all fumigation was discontinued, the two plots finally came to very close coincidence in their photosynthetic activity, the average value of the treated plot being 98 per cent. of the check during this period. The reduction in the amount of net assimilation on 28 days of treatment (not including September 17, 18, and 19) was 3.2 per cent. of the total net assimilation. As 16 per cent. of the latter was utilized for top growth, the estimated effect of these treatments on the yield of the crop was a reduction of about 0.5 per cent. The actual yields were practically identical. The drought certainly did not add to the effect of the fumigation treatment.

The sulphur and chlorophyll analyses on plots 5 and 6 are summarized in table XV. The chlorophyll content of the leaves decreased gradually, plot 6 a little more than plot 5, but not so much as the alfalfa outside the plant chambers. These differences in the chlorophyll content of the leaves are probably without practical significance.

It will be noted that the sulphur content of the leaves of plot 6 increased to about 1.7 per cent. The sulphur content of the upper leaves was always higher in plot 6 than that of the lower leaves, and the older upper leaves contained more sulphur than the younger upper leaves. In the check plot the lower leaves always had more sulphur than the upper leaves. The total excess of sulphur in plot 6 over plot 5, given by the analyses of plant tissue, is 7.44 gm. as compared with 5.91 gm. given by gas analyses in table XII. Again, this excess may be ascribed to an accumulation of this element from earlier fumigations. The amounts of sulphur present in the plant tissue on September 4 have been calculated, using an estimated value for the amount of plant substance present, which is based on comparable plants harvested on August 24 and on subsequent measurements of stem growth. When these sulphur values are subtracted from the total sulphur subsequently found, the excess of this element in plot 6 over plot 5 becomes 5.19 gm., as compared with 5.06 gm. determined by gas analyses. The latter value includes half of the sulphur absorbed on September 4, because the plant samples of September 4 were taken in the middle of the fumigation period. The concordance between these values is very close, but it is likely that plot 6 was being supplied with sulphur from its roots a little more rapidly than plot 5, even in this period, because there is always some absorption by the cabinet and the soil.

#### **Effect of very long continuous fumigations with sulphur dioxide of low concentration on carbon dioxide exchange**

Three series of long continuous fumigations with different low concentrations of sulphur dioxide—one of them lasting 45 days—were conducted



TABLE XIV  
BALANCE SHEET OF CARBON DIOXIDE EXCHANGE AND GROWTH OF ALFALFA PLOTS 5 AND 6—1936

DATE (1936)	TOTAL APPARENT ASSIMILATION CO <sub>2</sub>				NIGHT RESPIRATION CO <sub>2</sub>				NET ASSIMILATION CO <sub>2</sub>			
	PLOT 5 CHECK	PLOT 6 FUMI- GATED	RATIO 6/5		PLOT 5 CHECK	PLOT 6 FUMI- GATED	RATIO 6/5		PLOT 5 CHECK	PLOT 6 FUMI- GATED	RATIO 6/5	
August 24-29	gm.	gm.	%		gm.	gm.	%		gm.	gm.	%	
August 30-31*	900.3	910.3	101		82.7	86.7	105		817.6	823.6	101	
“ “			102 <sup>a</sup>									
“ “			96 <sup>b</sup>									
“ “			101 <sup>c</sup>									
September 1	303.3	298.9	99 <sup>d</sup>		40.8	38.8	95		262.5	260.1	99	
September 2-16*	186.3	192.0	103		19.2	15.4	80		167.1	176.6	106	
“ “			98 <sup>a</sup>									
“ “			94 <sup>b</sup>									
“ “			99 <sup>c</sup>									
September 17-19*	2755.5	2638.7	96 <sup>d</sup>		170.2	186.3	109		2585.3	2452.4	95	
“ “			101 <sup>a</sup>									
“ “			104 <sup>b</sup>									
“ “			107 <sup>c</sup>									
September 20-30*	490.9	516.4	105 <sup>d</sup>		23.1	29.1	126		467.8	487.3	104	
“ “			104 <sup>a</sup>									
“ “			83 <sup>b</sup>									
“ “			99 <sup>c</sup>									
October 1-4	1888.3	1816.8	96 <sup>d</sup>		93.6	108.7	116		1794.7	1708.1	95	
“ “	688.5	676.2	98		37.7	46.2	123		650.8	630.0	97	
Totals	7213	7049	98		467	511	109		6746	6538	97	
Equivalent dry matter (44 per cent. carbon) (grams)												
Dry matter October 5 at harvest (grams)												
Dry matter August 24 (estimated from weight of equivalent plot harvested August 24) (grams)												
Top growth during experiment (grams)												
Top growth—percentage of total CO <sub>2</sub> assimilated												
Probable root increment (grams)												
<div> <div>4180</div> <div>1480</div> <div>845</div> <div>635</div> <div>15.2</div> <div>3545</div> </div> <div> <div>4052</div> <div>1508</div> <div>845</div> <div>663</div> <div>16.4</div> <div>3389</div> </div>												
102%												

\* A 4.25-hour fumigation on plot 6 each morning on these dates, except September 2, 3, 10, and 11, when fumigation was given in the afternoon. <sup>a</sup> Before fumigation. <sup>b</sup> During fumigation. <sup>c</sup> After fumigation. <sup>d</sup> Total for the period.

TABLE XV  
LYSES OF PLOTS 5 AND 6 FOR SULPHUR AND CHLOROPHYLL (DRY BASIS)—1936

DATE (1936)	PLANT TISSUE	SULPHUR (S)					CHLOROPHYLL			
		PLOT 5 (HECK)			PLOT 6 (FUMIGATED)		PLOT 5 CHECK	PLOT 6 FUMI- GATED	OUTSIDE* CHECK	%
		WEIGHT OF TISSUE	SULPHUR (S)	WEIGHT (S)	WEIGHT OF TISSUE	SULPHUR (S)				
		gm.	%	gm.	gm.	%	%	%	%	%
September 4	Leaves	480 <sup>a</sup>	0.572	2.75	480 <sup>a</sup>	0.990	1.78	1.70	1.61	
" 4	Stems	550 <sup>a</sup>	0.199	1.09	553 <sup>a</sup>	0.242				
" 4	Total	1030	0.373	3.84	1035	0.588				
" 8	Leaves	18.0	0.566	0.102	21.2	1.168	1.83	1.74	1.36	
" 14	Upper leaves	22.2	0.485	0.108	21.5	1.400	1.75	1.67	1.37	
" 14	Lower "	7.3	0.775	0.057	6.5	0.860	1.72	1.66		
" 19	Upper leaves	20.6	0.463	0.095	21.0	1.647	1.56	1.27	1.29	
" 19	Lower "	7.4	0.630	0.047	9.9	1.418	1.61	1.48	1.60	
" 28	Upper leaves	22.8	0.437	0.100	24.9	1.758	1.51	1.28	1.05	
" 28	Lower "	6.0	0.595	0.036	8.6	1.510	1.67	1.39	1.22	
October 5	Upper secondary	235	0.450	1.058	232	1.670	1.42	1.36	1.29	
" 5	Upper primary	129	0.576	0.744	152	1.748	1.58	1.34		
" 5	Lower leaves	92	0.628	0.578	65	1.391	1.47	1.20	1.34	
September 4	Stems	21.1	0.206	0.043	28.0	0.269	0.28	0.31	0.31	
" 8	"	43.3	0.156	0.068	42.4	0.238	0.27	0.27	0.28	
" 14	"	41.8	0.168	0.070	41.0	0.238	0.30	0.32		
" 19	"	43.8	0.166	0.073	47.8	0.265	0.26	0.31		
" 28	"	640	0.130	0.831	652	0.250	0.26	0.26		
October 5	Dead tissue	72	0.405	0.292	87	0.710	0.34	0.38	0.31	
" 5	Leaves and stems		0.293			0.824				
Total sulphur September 8 to October 5		430							11.74	
Less: sulphur of September 4		3.84							6.09	
Increment of sulphur September 8 to October 5		0.46							5.65	
Excess of sulphur in plot 6 over plot 5									7.44	
Excess of sulphur in plot 6 over plot 5 after September 4									5.19	

\* Comparable alfalfa grown adjacent to and outside of plant chambers.      \* Estimated.

TABLE XVI  
FUMIGATION DATA OF PLOT 8—1935

DATES	DURATION	AVERAGE AIR VELOCITY EACH DAY				AVERAGE SO <sub>2</sub> CONCENTRATION EACH DAY				WEIGHT OF SO <sub>2</sub> ABSORBED EACH DAY	
		INTAKE		OUTLET							
						DAY	NIGHT	DAY	NIGHT	DAY	NIGHT
September 18 to October 10	528 Hours or 22 Days	Minimum*	<i>l.p.m.</i>	<i>l.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>gm.</i>	<i>gm.</i>
		3260	545	0.217	0.149	0.072	0.062	0.462	0.101		
		Maximum*	945	0.254	0.276	0.135	0.171	0.683	0.224		
		Average	3347	766	0.232	0.240	0.114	0.112	0.581	0.173	
Average percentage of absorption											
Average rate of absorption (mg. SO <sub>2</sub> per minute per 1000 gm. of leaves)											
										51%	53%
										1.4	0.38
Total SO <sub>2</sub> absorbed (grams)											
										16.6	
Total S absorbed (grams)											
										8.3	

\* Maximum and minimum values in this table were taken from many different fumigations.

at Logan, Utah, in 1935, for the purpose of determining the maximum effects such concentrations could have under the most drastic conditions of application.

G. EFFECT OF CONTINUOUS FUMIGATION AT 0.24 P.P.M.—Plots 7 and 8 were under observation from September 12 to October 15, 1935. The variety of alfalfa in these plots was Utah Common. The fumigation data for plot 8 are given in table XVI. The plot was fumigated continuously from September 18 to October 10—528 hours, or about 22 days. The average intake concentration was 0.236 p.p.m., the average outlet was 0.113 p.p.m., and the mean was 0.175 p.p.m.

The total daily values of apparent assimilation and respiration for plots 7 and 8 are presented in figure 7, and the data are summarized in table XVII. Detailed carbon dioxide exchange curves are given in figure 8, charts A, B, and C. It will be noted that the treated plot was somewhat more vigorous than the check plot at the beginning of the experiment. Accordingly, the ratios of the carbon dioxide values for the two plots have been adjusted to 100 per cent. for the period immediately preceding the fumigation treatment in order to facilitate the comparison of the two plots. During the first 3 days of the fumigation treatment, the rate of apparent assimilation of the treated plot was 1 per cent. less than the comparable rate before the beginning of the treatment. Then the rate fell to 97 per cent. of the check for the next 3 days, to 88 per cent. for 3 days, to 85 per cent. for 7 days, and during the last 6 days of the fumigation to 76 per cent. Finally, the activity rose to a level of about 81 per cent. of the check in the period following the fumigation.

On the treated plot a few bleached markings were first noted 8 days after the fumigation commenced. It was also noted that the older primary leaves were becoming chlorotic. During the next 10 days no new acute markings were found, but many of the older leaves became more chlorotic, until they finally dried up and dropped off. The check plot was nearly free of this type of lesion. During the last 4 days of the fumigation, more acute markings were produced, being generally distributed over the plot. At harvest, plot 8 had 1.7 per cent. of acute markings and 7 per cent. of chlorotic markings. The older primary leaves had 12 per cent. of chlorotic markings. The leaves of the check plot had 6 per cent. of chlorotic discoloration, but no dead lesions. Weather conditions were doubtless responsible for the discolorations on the check plot and for about an equal amount of lesions on the treated plot, leaving only the acute markings and some of the chlorotic markings, particularly on the older leaves, to be ascribed to the fumigation. It will be observed in table XVII that at harvest time, October 16, the weight of functioning leaves on the check plot was 502 gm. as compared with 428 gm. on the treated plot. This reduction in the weight

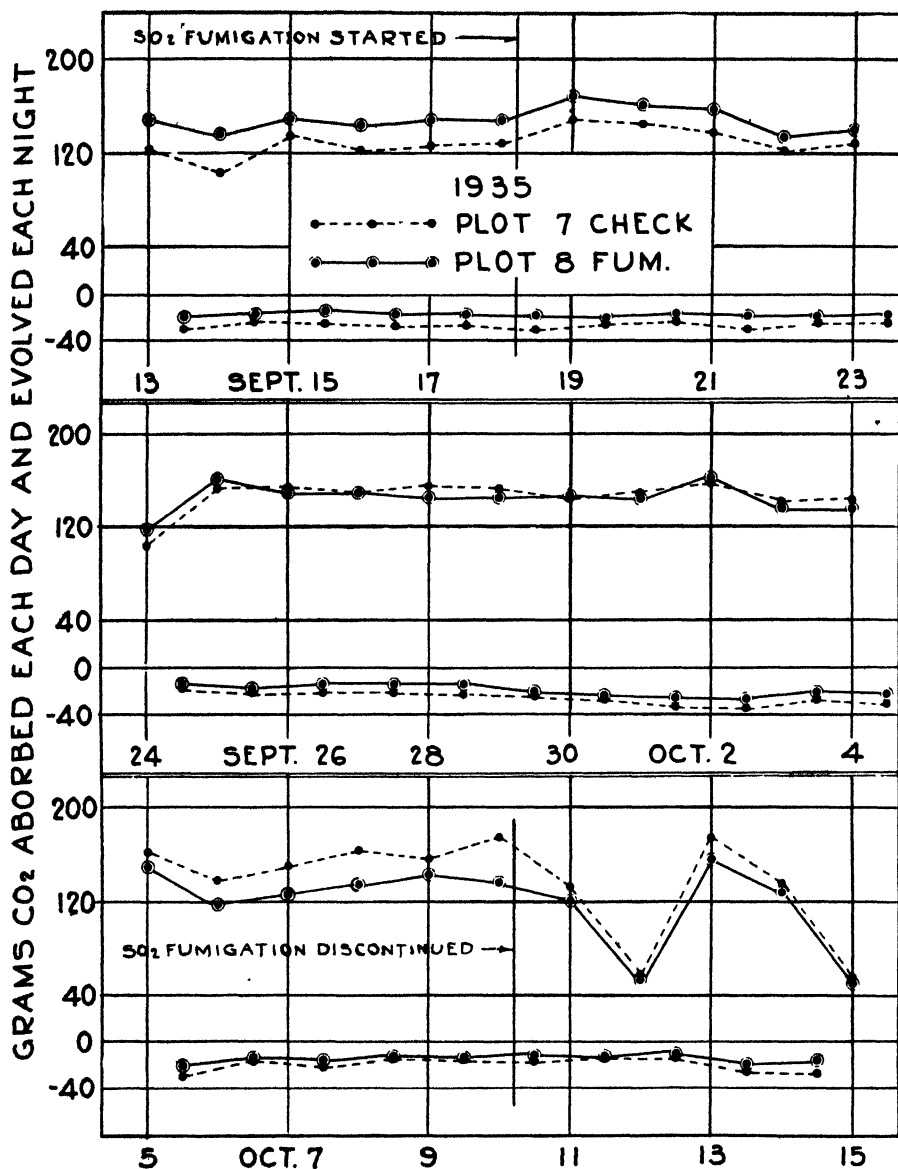


FIG. 7. Total daily apparent assimilation and respiration of plots 7 and 8 at Logan, Utah (1935). Plot 8 was fumigated continuously from September 18 to October 10 (22 days) with 0.235 p.p.m. SO<sub>2</sub>.

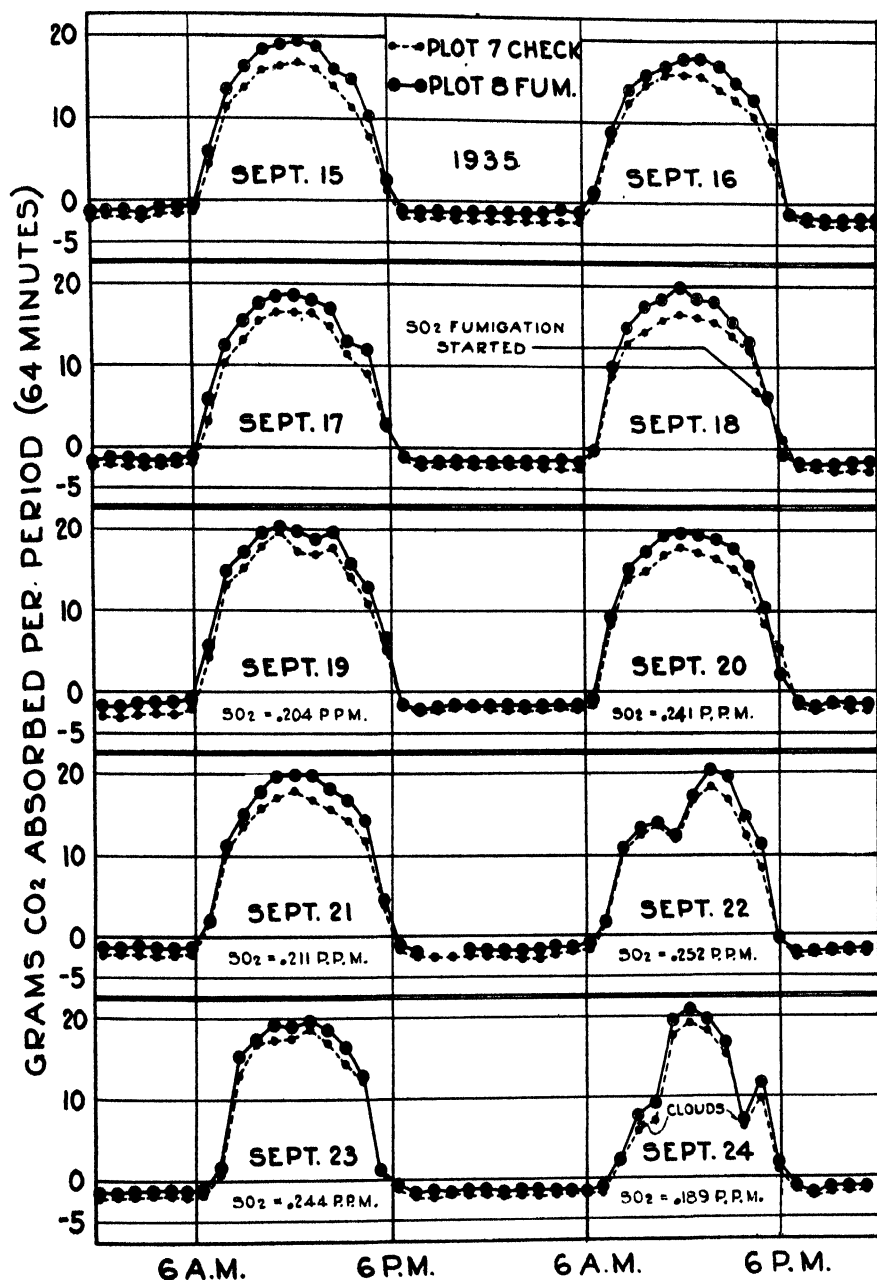


FIG. 8. Chart A, September 15-24, 1935. Apparent assimilation and respiration of plots 7 and 8 at Logan, Utah. Plot 8 was fumigated continuously from September 18 to October 10 (22 days) with 0.235 p.p.m. SO<sub>2</sub>.

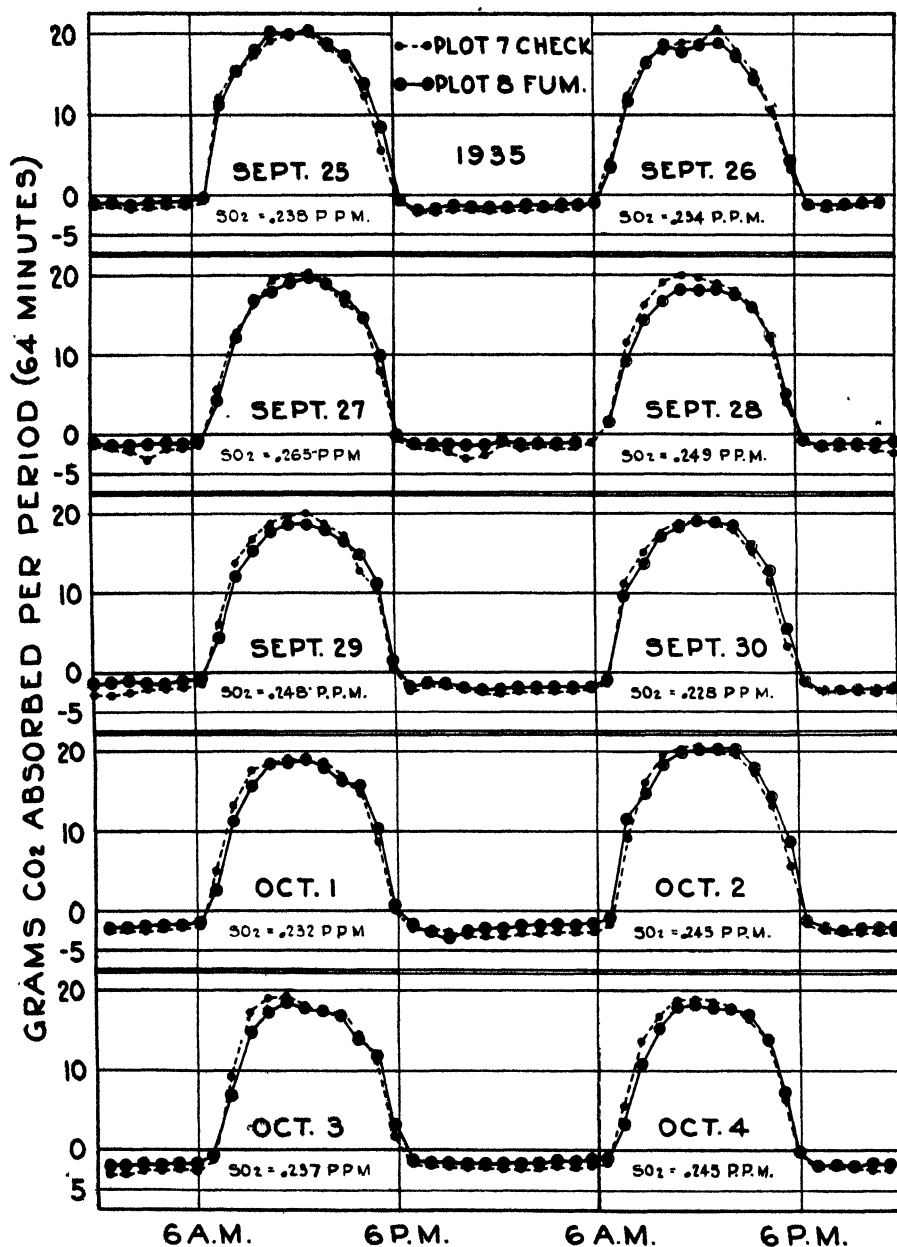


FIG. 8. Chart B, September 25–October 4, 1935.

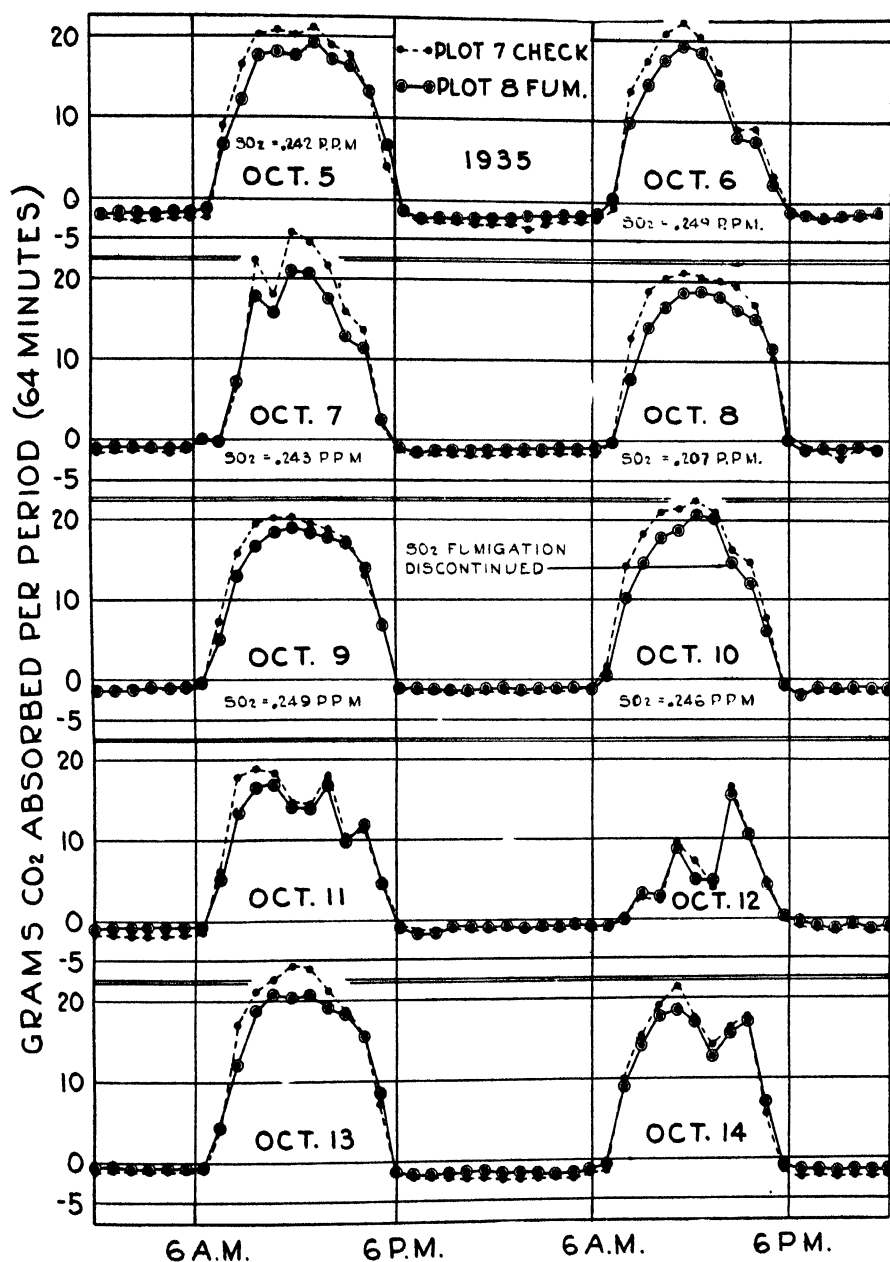


FIG. 8. Chart C, October 5-14, 1935.



TABLE XVII

# BALANCE SHEET OF CARBON DIOXIDE EXCHANGE AND GROWTH OF ALFALFA

### PLOTS 7 AND 8—1935

DATES (1935)	TOTAL APPARENT ASSIMILATION CO <sub>2</sub>				NIGHT RESPIRATION CO <sub>2</sub>				NET ASSIMILATION CO <sub>2</sub>			
	PLOT 7 CHECK	PLOT 8 FUMI- GATED	RATIO 8/7		PLOT 7 CHECK	PLOT 8 FUMI- GATED	RATIO 8/7		PLOT 7 CHECK	PLOT 8 FUMI- GATED	RATIO 8/7	
			ACTUAL	ADJUSTED			ACTUAL	ADJUSTED			ACTUAL	ADJUSTED
gm.	gm.	%	%	gm.	gm.	%	%	gm.	gm.	%	%	
9/13- 9/15	365	438	120	105	74	47	64	100	291	391	134	105
9/16- 9/18	380	442	116	102	54	35	65	101	326	407	125	98
9/18	.....	.....	114	100	.....	.....	64	100	.....	.....	128	100
Continuous fumigation started at 5:06 P.M. Sept. 18—average SO <sub>2</sub> concentration 0.236 p.p.m.												
9/19- 9/21	434	490	113	99	107	71	66	103	327	419	128	100
9/22- 9/24	356	394	111	97	69	50	72	113	287	344	120	94
9/25- 9/27	460	461	100	88	61	44	72	113	399	417	105	82
9/28-10/ 4	1058	1028	97	85	194	144	74	116	864	884	102	80
10/ 5-10/10	925	804	87	76	118	91	77	120	807	713	88	69
Average	.....	.....	98	86	.....	.....	73	114	.....	.....	103	81
10/11-10/14	504	464	92	81	79	56	71	111	425	408	96	75
Totals	4482	4521	101	89	756	538	71	111	3726	3983	107	84
Fumigation discontinued at 2:48 P.M. Oct. 10												
Equivalent dry matter (grams) (44 per cent. carbon)												
Functioning tissue (grams)												
Samples for analysis October 3												
Harvest October 16												
Dead tissue October 16 (grams)												
Total tissue to October 16 (grams)												
Dry matter September 12 (estimated, grams)												
Top growth during experiment (grams)												
Top growth—percentage of total CO <sub>2</sub> assimilated												
Probable root increment (grams)												
<div>Plot 7</div> <div>Plot 8</div>												
<div>2312</div> <div>2470</div>												
<div>40<sup>a</sup></div> <div>32<sup>a</sup></div> <div>49<sup>b</sup></div> <div>50<sup>b</sup></div> <div>502<sup>a</sup></div> <div>682<sup>b</sup></div> <div>172</div> <div>320</div>												
<div>1446</div> <div>644</div> <div>802</div> <div>34.7</div> <div>1510</div>												
<div>1483</div> <div>715</div> <div>768</div> <div>34.1</div> <div>1702</div>												

**a Leaves.** **b Stems.**

**Stems,**

of functioning leaves, together with the greater amount of leaf area marked on the leaves that were left, and perhaps also their somewhat lowered chlorophyll content, indicated later, can account for the reduced photosynthetic activity. The estimated reduction of apparent assimilation which can be ascribed to the fumigation treatment is 11 per cent. of the total apparent assimilation, or 16 per cent. of the total net assimilation. The latter is equivalent to a reduction in top growth of about 8 per cent., since in this experiment only one-third of the net assimilation was converted into top growth. Slightly more top growth was actually found on plot 8 than on plot 7, due perhaps to the more vigorous activity noted at the beginning of the experiment.

Figure 7 and table XVII show a rather large and consistent difference in the rate of respiration of the two plots. This difference is also a noticeable feature of the two following 1935 experiments, whereas in the 1933 and 1936 experiments the pairs of plots exhibited consistently close agreement. The reason for the differences in respiration noted in 1935 is not known. As pointed out in the previous paper (8) no account was taken in this work of "soil respiration", though an appreciable and varying amount is known to exist. The plantings in 1935 were only one year old, and there is a possibility that the rate of "soil respiration" was different on the pairs of plots at that time, due perhaps to unequal amounts of undecomposed manure, but subsequently became equal. It may also be noted that in 1935, the remains of the preceding crop had not been cleared off as thoroughly as in 1936, suggesting the possibility of unequal amounts of decomposition of organic matter on the surface of the soil in 1935. Whatever the cause of the differences, they were sufficiently consistent that they do not present serious difficulties in the interpretation of the photosynthetic data.

Detailed sulphur and chlorophyll analyses were made on the leaves and stems on October 3, and at harvest, October 16 (table XVIII). The leaves were separated into three groups according to age. The sulphur content of the check leaves increased with age. On October 3 the intermediate leaves on the treated plot had the most sulphur, probably because the lower primary leaves were somewhat protected from the gas by the upper leaves which absorbed part of the sulphur dioxide and reduced the concentration of the gas which reached the lower leaves. By October 16 many of the lower leaves had been shed and the remaining primary leaves had the most sulphur. Both the primary and secondary leaves carried about 2 per cent. of sulphur, and the chlorophyll was appreciably lower than in the corresponding check leaves. A sulphur content greater than 2.5 per cent. has never been observed. The tertiary leaves on the other hand with 1.3 per cent. of sulphur were apparently normal in chlorophyll content. The

TABLE XVIII  
ANALYSES OF PLOTS 7 AND 8 FOR SULPHUR AND CHLOROPHYLL—1935

DATE (1935)	PLANT TISSUE	SULPHUR (S)					CHLOROPHYLL			
		PLOT 7 (CHECK)			PLOT 8 (FUMIGATED)		PLOT 7 CHECK	PLOT 8 FUMI- GATED	OUTSIDE* CHECK	
		WEIGHT OF TISSUE	SULPHUR (S)	WEIGHT (S)	WEIGHT OF TISSUE	SULPHUR (S)				WEIGHT (S)
October 3	Tertiary leaves	gm.	%	gm.	gm.	%	%	%	%	
" 3	Secondary "	17.6	0.414	0.073	13.9	1.216	0.168	1.24	1.19	1.01
" 3	Primary "	12.8	0.505	0.065	12.0	1.766	0.212	1.36	1.04	1.14
October 16	Tertiary leaves	9.3	0.591	0.055	6.0	1.600	0.096	1.38	0.91	1.30
" 16	Secondary "	180	0.434	0.781	168	1.315	2.209	1.29	1.21	1.39
" 16	Primary "	197	0.517	1.018	173	1.936	3.349	1.33	1.11	1.34
" 16	Dead "	125	0.633	0.791	87	2.130	1.853	1.31	0.92	1.38
October 3	Stems	82	0.583	0.478	160	0.933	1.493			
" 16	"	49.7	0.173	0.086	48.7	0.250	0.122			
" 16	Dead stems	682	0.192	1.309	654	0.278	1.818			
" 16	Leaves and stems	90	0.190 <sup>a</sup>	0.171	160	0.250 <sup>a</sup>	0.400			
			0.329			0.852				
Total				4.83			11.72			
Excess of S. in plot 8 over plot 7							6.89			

\* Comparable alfalfa grown adjacent to and outside of plant chambers.

<sup>a</sup> Estimated.

TABLE XIX

FUMIGATION DATA OF PLOT 6—1935

DATES (1935)	DURATION	AVERAGE AIR VELOCITY EACH DAY				AVERAGE SO <sub>2</sub> CONCENTRATION EACH DAY				WEIGHT OF SO <sub>2</sub> ABSORBED EACH DAY	
		DAY		NIGHT		INTAKE		OUTLET		DAY	NIGHT
August 26 to September 21	628 hours or 26 days	Minimum* Maximum* Average	<i>l.p.m.</i> 3765 4150 3960	<i>l.p.m.</i> 690 857 768	<i>p.p.m.</i> 0.152 0.218 0.188	<i>p.p.m.</i> 0.143 0.211 0.187	<i>p.p.m.</i> 0.047 0.111 0.077	<i>p.p.m.</i> 0.041 0.135 0.090	<i>gm.</i> 0.505 0.922 0.675	<i>gm.</i> 0.089 0.185 0.128	
Average percentage of SO <sub>2</sub> absorbed August 26–September 21											
Average rate of absorption (mg. SO <sub>2</sub> per minute per 1000 gm. of leaves)											
September 25 to October 9	336 hours or 14 days	Minimum* Maximum* Average	3713 3957 3880	623 828 740	0.258 0.311 0.292	0.234 0.315 0.290	0.107 0.185 0.147	0.095 0.185 0.144	0.683 1.000 0.829	0.155 0.234 0.198	
Average percentage of SO <sub>2</sub> absorbed September 25–October 9											
Average rate of absorption (mg. SO <sub>2</sub> per minute per 1000 gm. of leaves)											
										50% 2.2	50% 0.43
SO <sub>2</sub> absorbed August 26–September 21 (grams)											
SO <sub>2</sub> absorbed September 25–October 9 (grams)											
Total SO <sub>2</sub> absorbed (grams)											
Total S absorbed (grams)											
										21.12 14.37 35.49 17.74	

\* The maximum and minimum values in the horizontal lines of this table occurred in different fumigations and are, therefore, unrelated.

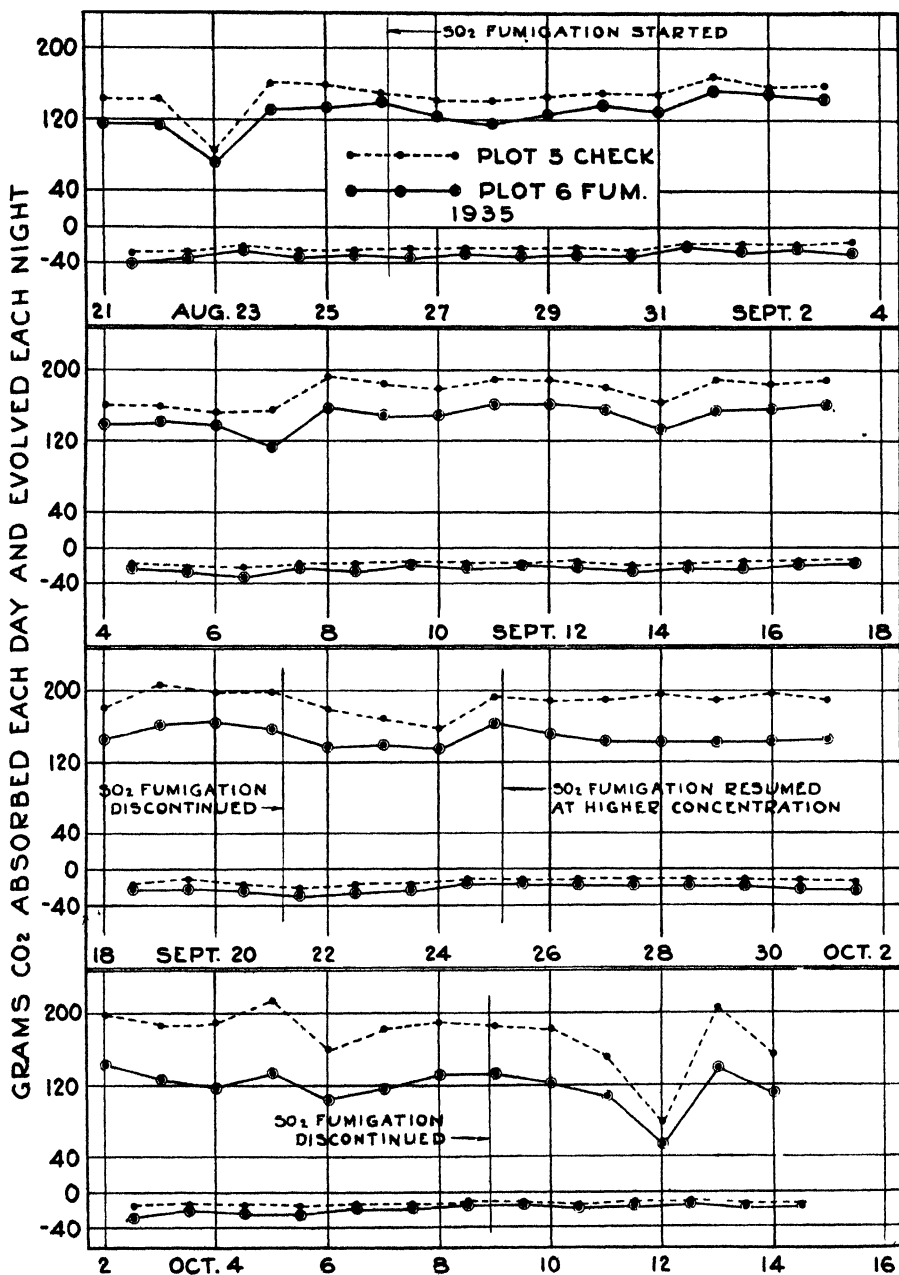


FIG. 9. Total daily apparent assimilation and respiration of plots 5 and 6 at Logan, Utah (1935). Plot 6 was fumigated continuously from August 26 to September 21 (26 days) with 0.19 p.p.m. SO<sub>2</sub>, and continuously from September 25 to October 9 with 0.29 p.p.m. SO<sub>2</sub>.

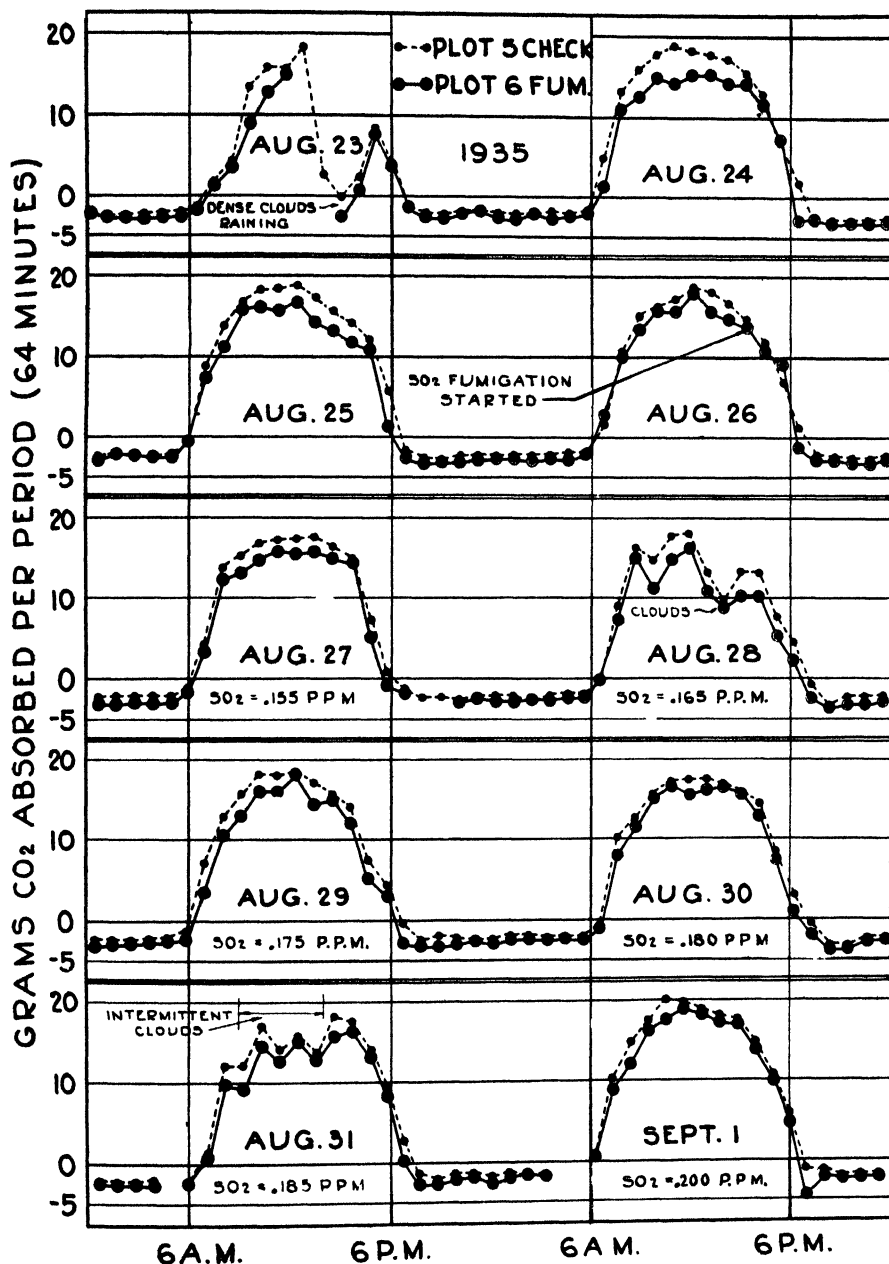


FIG. 10. Chart A, August 23–September 1, 1935. Apparent assimilation and respiration of plots 5 and 6 at Logan, Utah. Plot 6 was fumigated continuously from August 26 to September 21 (26 days) with 0.19 p.p.m. SO<sub>2</sub> and continuously from September 25 to October 9 with 0.29 p.p.m. SO<sub>2</sub>.

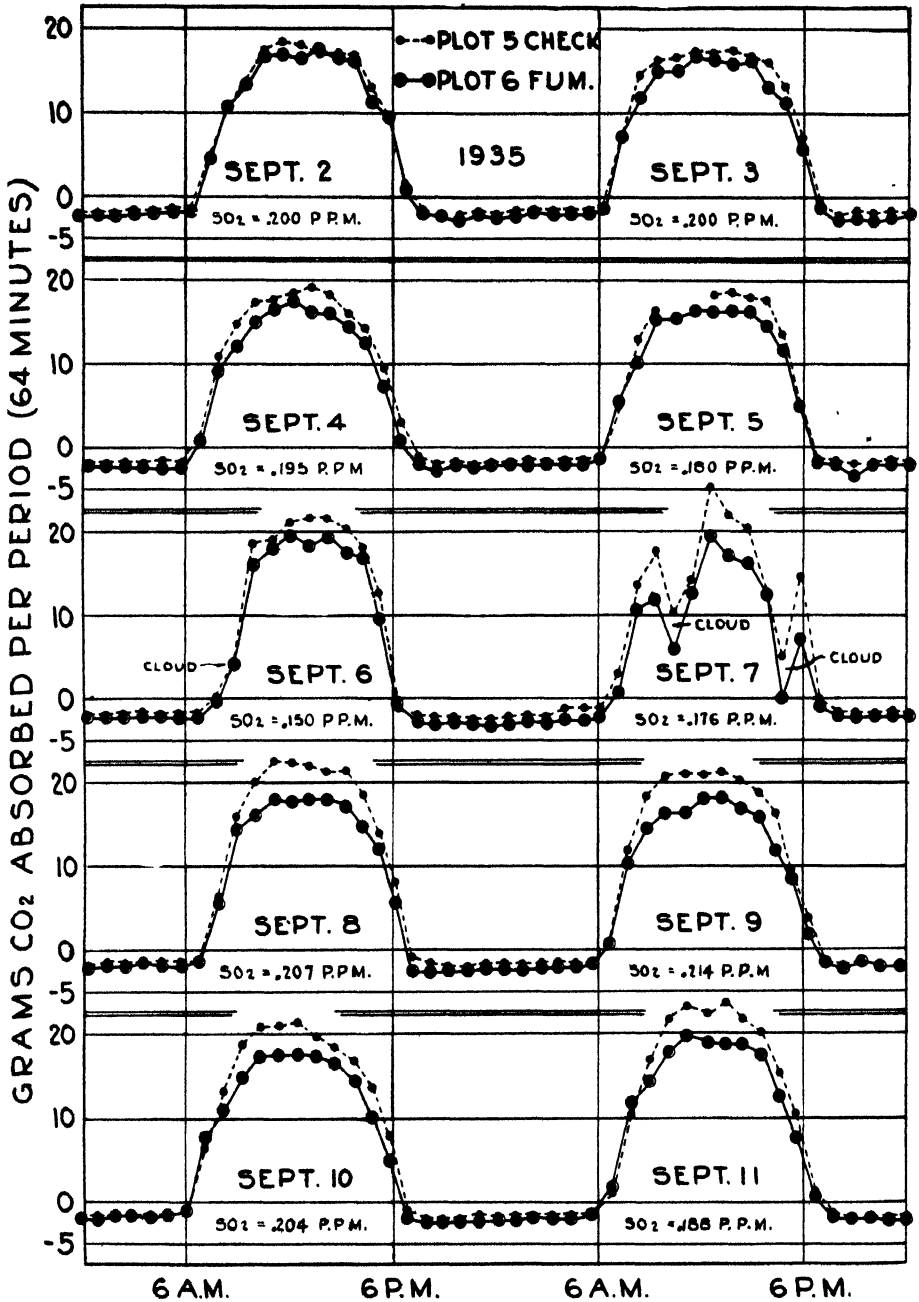


FIG. 10. Chart B, September 2-11, 1935.

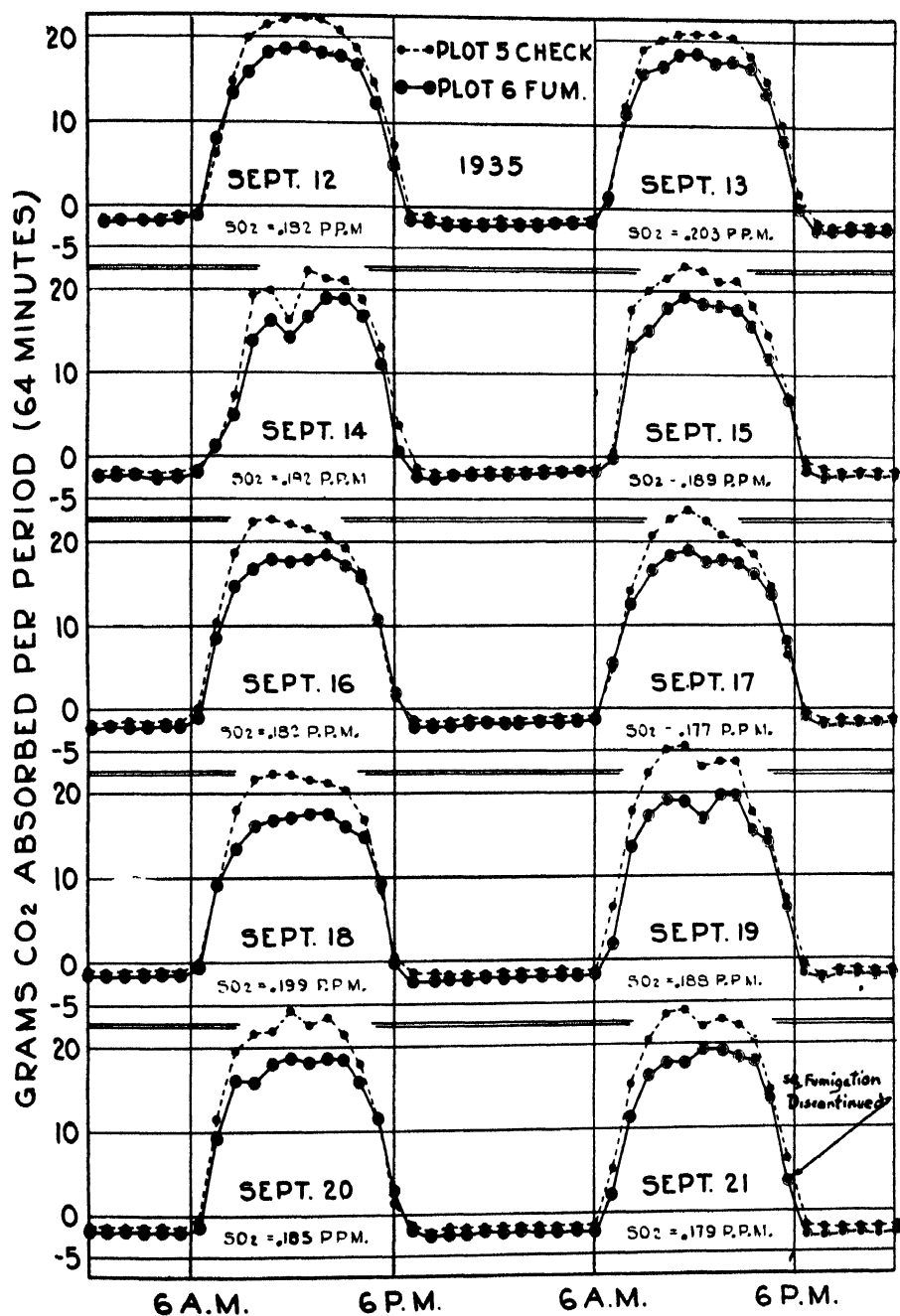


FIG. 10. Chart C, September 12-21, 1935.



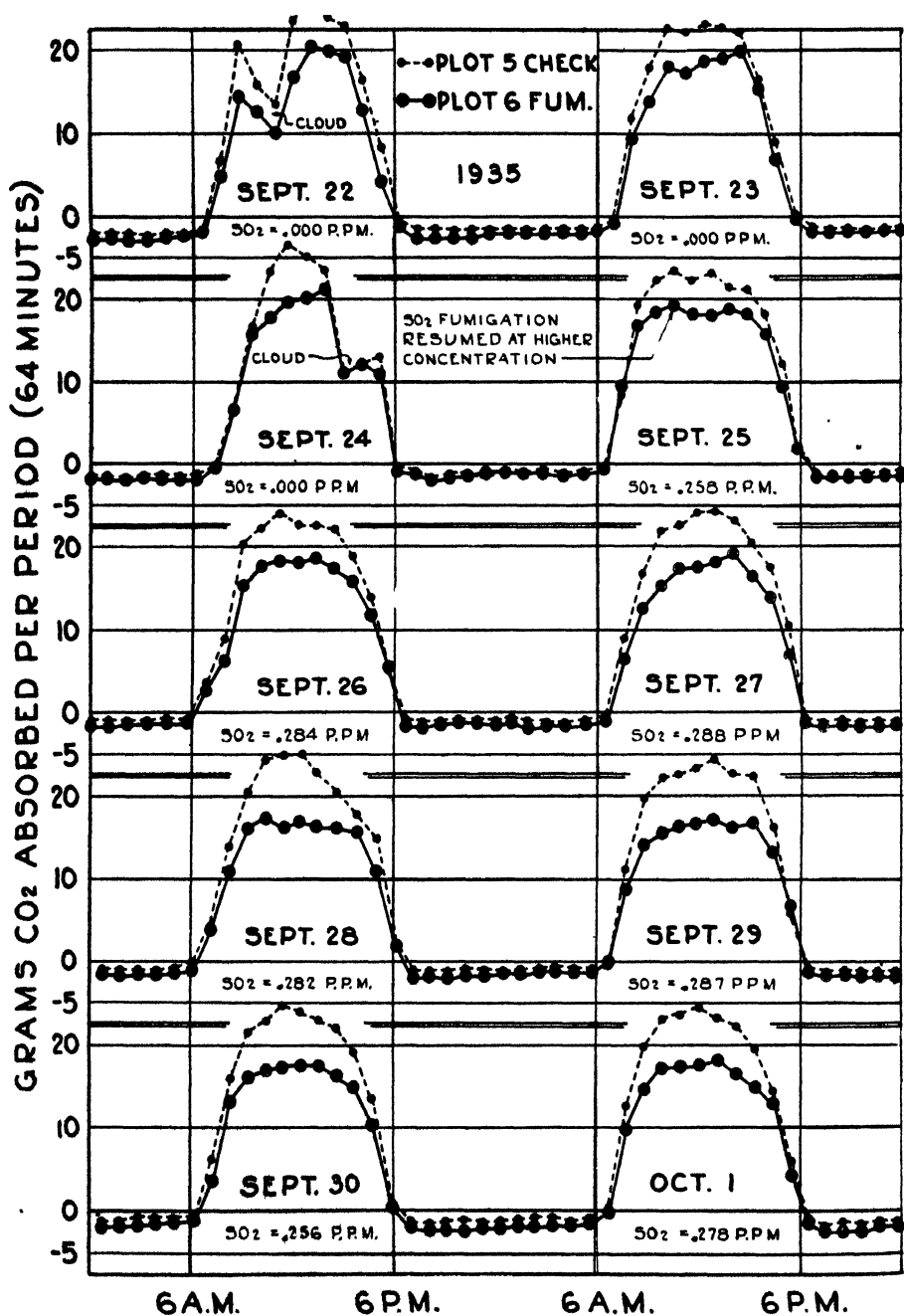


FIG. 10. Chart D, September 22–October 1, 1935.

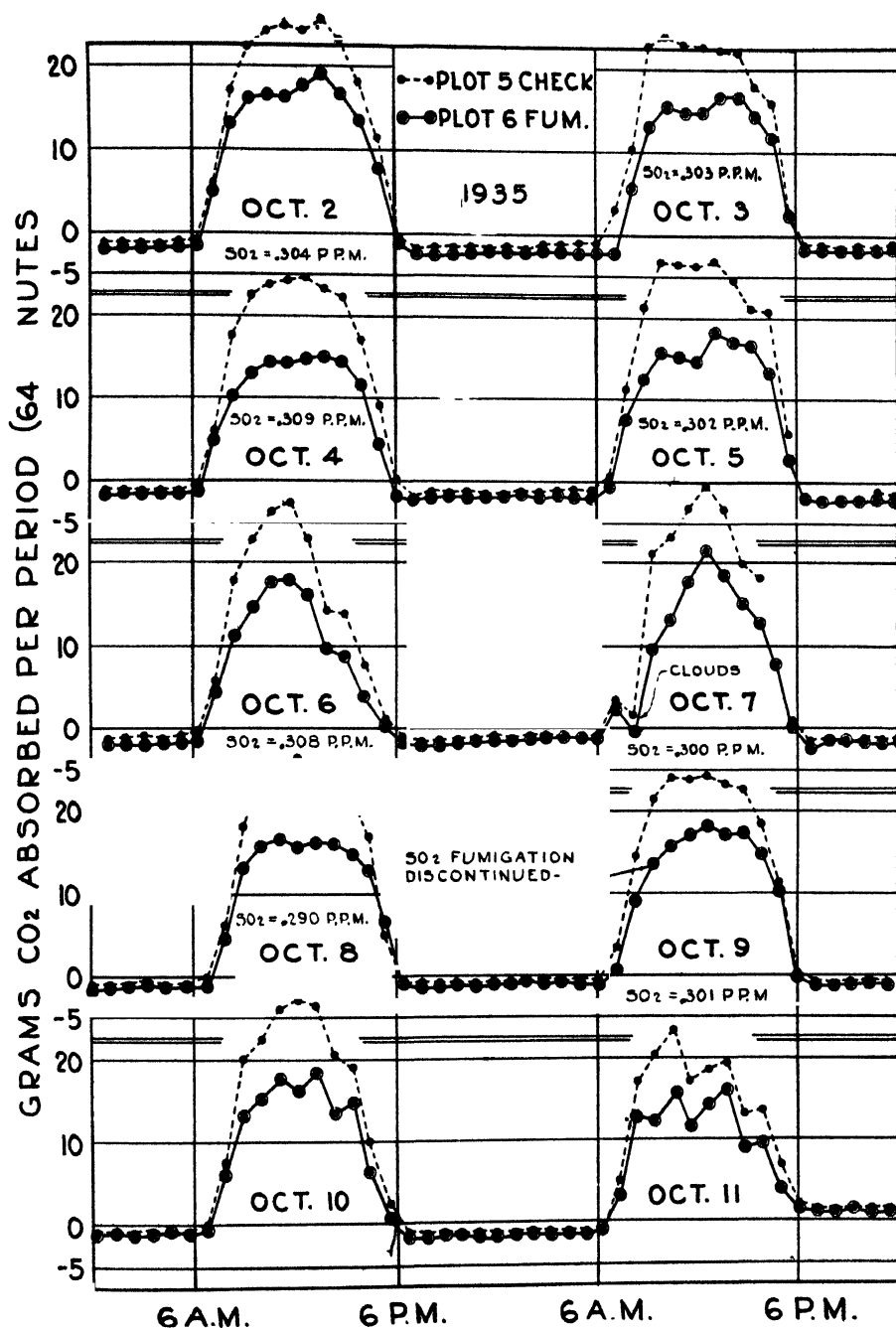


Fig. 10. Chart E, October 2-11, 1935.

excess of sulphur in the plant tissue of plot 8 over plot 7 was 6.90 gm., as compared with 8.3 gm. absorbed as determined by gas analyses.

H. EFFECT OF A CONTINUOUS FUMIGATION OF ABOUT 0.2 P.P.M. FOR ABOUT 26 DAYS FOLLOWED BY A CONTINUOUS FUMIGATION OF ABOUT 0.3 P.P.M. FOR ABOUT 14 DAYS.—Plots 5 and 6 were under observation from August 21 to October 15, 1935. These are the same 2 plots as are described earlier in connection with the fourth 1936 experiment, in which they were given the same plot numbers. The fumigation data are summarized in table XIX. The plot was fumigated continuously from August 26 to September 21—628 hours or about 26 days—with an average intake concentration of 0.188 p.p.m. of  $\text{SO}_2$ , an average outlet concentration of 0.083 p.p.m., and a mean concentration of 0.136 p.p.m. Then following a rest period of 4 days, the fumigation was resumed from September 25 to October 9—336 hours or about 14 days—with an average intake concentration of 0.291 p.p.m., an average outlet of 0.145 p.p.m., and a mean of 0.218 p.p.m.

The total daily apparent assimilation and respiration of the 2 plots are presented in figure 9, and the data are summarized in table XX. Detailed curves of carbon dioxide exchange are given in figure 10 charts A, B, C, D, and E. As the treated plot exhibited a lower rate of apparent assimilation, and a higher rate of respiration, before the fumigation treatment was applied, the values of the ratios have again been adjusted to facilitate the comparison of the 2 plots. It will be noted that the fumigation caused no reduction in the rate of photosynthesis during the first 11 days. There was actually an increase of about 1 per cent. in the net assimilation. Then the apparent assimilation level fell to 89 per cent. of the check for 3 days, then rose to 95 per cent. for the next 8 days, and finally fell to 90 per cent. during the last 4 days of the first fumigation treatment. During the rest period the level of apparent assimilation rose to 95 per cent. of the check. With the administration of the higher concentration, the level of the treated plot gradually fell until finally it was 73 per cent. of the check. After the fumigation was finally discontinued the photosynthetic level of the treated plot rose to about 79 per cent. of the check.

During the first fumigation period only a few markings were observed on the treated plot, and these were largely confined to the older leaves, which seemed to become yellow and drop off somewhat earlier than on the check plot. After the fumigation was resumed at the higher concentration, however, acute and chlorotic markings were conspicuous on the treated plot and they became progressively more numerous as the fumigation progressed. At harvest all of the leaves were separated from the stems and the leaves were collected into 3 groups, according to age, as primary, secondary, and tertiary. The oldest leaves on plot 6 had 2.4 per cent. acute markings and 12.4 per cent. of chlorotic markings. The average values for the plot were

**BALANCE SHEET OF CARBON DIOXIDE EXCHANGE AND GROWTH OF ALFALFA PLOTS 5 AND 6—1935**

**a Leaves.**                      **b Stems.**

1.2 per cent. of acute, and 5.5 per cent. of chlorotic markings. The latter were partly weather discolorations, because plot 5 had about 2 per cent. of the same type, but no bleached discolorations. The amount of marked leaf area remaining on the plants at harvest time is insufficient to account for the observed reduction of about 21 per cent. in the final rate of assimilation. Table XX indicates that there were only 511 gm. of functioning leaves on the treated plot at harvest, as compared with 597 gm. on the check plot. As the weights of stems on the two plots were more nearly equal, the difference between the weights of the leaves probably represents largely leaves that were shed. The reduced amount of functioning leaves with their discolorations and lowered chlorophyll content (table XXI) accounts for the reduced rate of assimilation.

Table XX indicates that during the first fumigation, the rate of net assimilation was reduced an average of 4 per cent., and during the second fumigation 21 per cent. The total reduction for the whole experiment was 10 per cent.

Sulphur and chlorophyll analyses are presented in table XXI. The values and relations of these constituents are quite similar to those of the preceding experiment. A smaller portion (9.79 out of 17.74 gm., tables XXI and XIX) of the total sulphur absorbed in plot 6 was accounted for by these analyses than was accounted for in plot 8. This is probably due to the fact that the fumigation of plot 6 was more protracted than that of plot 8. Some of the absorbed sulphur evidently went into the roots, because in the 1936 experiments, more sulphur was accounted for than was added later. It seems worth while to tabulate the sulphur balance on this crop and the three 1936 crops. This has been done in table XXII, which also includes the analyses of the roots which were dug up in a dormant condition in February, 1937. The air analyses show that in three fumigation experiments, in 1935 and 1936, 24.62 gm. of sulphur were added to plot 6 as sulphur dioxide. The analyses of the plant tissue from the fumigated crops in 1935 and 1936, plus that of the first crop of 1936 which was not fumigated, indicate that plot 6 had 21.59 gm. of sulphur more than plot 5. Each crop on plot 6 in 1936 returned some of the sulphur that was unaccounted for in 1935. There had evidently been a translocation in 1935 of a considerable portion of the added sulphur to the roots. There was still an excess of 0.36 gm. in the roots of plot 6 at the end of the 1936 season. The sulphur finally unaccounted for represents 12 per cent. of the total amount of sulphur dioxide added. This is about the same as the percentage unaccounted for in the other experiments in this paper, which were not complicated by previous fumigations. The soil contained 5.3 p.p.m. of sulphur as soluble sulphate in plot 5 and 5.1 p.p.m. in plot 6. No significant difference could be detected between soil of the two plots, even in the surface

TABLE XXI  
ANALYSES OF PLOTS 5 AND 6 FOR SULPHUR AND CHLOROPHYLL—1935

DATE (1935)	PLANT TISSUE	SULPHUR (S)						CHLOROPHYLL		
		PLOT 5 (CHECK)			PLOT 6 (FUMIGATED)			PLOT 5 CHECK	PLOT 6 FUMI- GATED	OUT- SIDE* CHECK
		WEIGHT OF TISSUE	SULPHUR (S)	WEIGHT (S)	WEIGHT OF TISSUE	SULPHUR (S)	WEIGHT (S)			
		gm.	%	gm.	gm.	%	gm.	%	%	%
September 10	Leaves	14.5	0.395	0.057	13.1	1.120	0.147	1.32	1.23	1.18
" 17	"	35.2	0.389	0.137	32.4	1.390	0.450	1.33	1.17	1.12
" 25	"	38.2	0.396	0.151	32.7	1.330	0.435	1.30	1.16	1.10
October 15	Tertiary leaves	238	0.365	0.869	175	1.628	2.852	1.37	1.00	1.04
" 15	Secondary "	242	0.421	1.019	261	1.972	5.147	1.26	1.01	1.03
" 15	Primary "	117	0.511	0.598	75	2.265	1.699	.....	.....	.....
" 15	Dead leaves	80	0.578	0.462	100	1.276	1.276	.....	.....	.....
September 10	Stems	18.8	0.165	0.031	23.2	0.198	0.046	.....	.....	.....
" 17	"	50.3	0.106	0.053	46.6	0.266	0.124	.....	.....	.....
" 25	"	52.1	0.162	0.084	46.4	0.186	0.086	.....	.....	.....
October 15	"	766	0.141	1.080	724	0.258	1.868	.....	.....	.....
" 15	Dead stems	200	0.140 <sup>a</sup>	0.280	240	0.20 <sup>a</sup>	0.480	.....	.....	.....
" 15	Leaves and stems	.....	0.262	.....	.....	0.937	.....	.....	.....	.....
Total		4.821			14.610			9.79		
Excess of S in plot 6 over plot 5		.....			.....			.....		

\* Comparable alfalfa grown adjacent to and outside of plant chambers.

<sup>a</sup> Estimated.

TABLE XXII

BALANCE SHEET OF SULPHUR IN PLOT 6 (FUMIGATED IN 1935 AND 1936) AS COMPARED WITH PLOT 5 (CHECK)

DATE OF HARVEST	PLANT TISSUE	SO <sub>2</sub> ADDED TO PLOT 6 (GAS ANALYSIS) (S)	TOTAL SULPHUR (S) IN TISSUE			EXCESS OF SULPHUR IN PLOT 6 OVER PLOT 5, MINUS SULPHUR ADDED AS SO <sub>2</sub>
			PLOT 5	PLOT 6	DIFFERENCE	
		<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
October 15, 1935	Third crop	17.74	4.82	14.61	9.79	- 7.95
June 26, 1936	First crop	None	6.22	7.50	1.28	+ 1.28
August 3, 1936	Second crop	0.97	5.47	8.19	2.72	+ 1.75
October 15, 1936	Third crop	5.91	4.30	11.74	7.44	+ 1.53
February 7, 1937	Roots		5.58	5.94	0.36	+ 0.36
Totals		24.62	26.39	47.98	21.59	- 3.03

inch. In preparing the roots for analysis, they were washed with distilled water. Soil, approximately equal in weight to the roots, was thus removed. This adhering soil from each plot contained 74 p.p.m. of sulphur as sulphate, which represents about 14 times as much soluble sulphate in the soil clinging to the roots as in the average soil of the plot. This suggests a passage outward of the sulphate from the roots to soil.

I. EFFECT OF A CONTINUOUS FUMIGATION OF 0.14 P.P.M. FOR ABOUT 45 DAYS.—Plots 3 and 4 were under observation from July 16 to September 5, 1935. These were the same plots that were described above in connection with a 1936 experiment. The fumigation data for plot 4 are summarized in table XXIII. The plot was given a continuous treatment with sulphur dioxide from July 20 to September 3—1078 hours or about 45 days. The average intake concentration was 0.141 p.p.m. and the average outlet concentration 0.050 p.p.m., or a mean of 0.095 p.p.m.

The total daily values of the apparent assimilation and respiration on these plots are presented in figure 11, and are summarized in table XXIV. The detailed daily charts for this experiment are given in figure 12 charts A, B, C, D, and E. The curves of photosynthesis indicate that plot 4 was somewhat less active than plot 3 before the fumigation treatment started, and again the ratios have been adjusted to facilitate comparison. In this case the initial fumigation period was used as reference, because two days in the prefumigation period, July 18 and 19, had almost as high an assimilation rate as obtained after July 21. The machines did not operate smoothly on July 20, and the differences noted on that date are probably too great. During the first 8 days of the fumigation, the rate of apparent assimilation of the treated plot was somewhat greater in relation to the check plot than

TABLE XXIII

FUMIGATION DATA OF PLOT 4—1935

DATES	DURATION	AVERAGE AIR VELOCITY EACH DAY				AVERAGE SO <sub>2</sub> CONCENTRATION EACH DAY						WEIGHT OF SO <sub>2</sub> ABSORBED EACH DAY			
				DAY		NIGHT		INTAKE		OUTLET					
July to September	20 1078 hours or 45 days	Minimum*	<i>l.p.m.</i>	731	<i>p.p.m.</i>	0.09	0.07	<i>p.p.m.</i>	0.017	<i>p.p.m.</i>	0.015	<i>gm.</i>	0.066		
		Maximum*		1330		0.19	0.26		0.073		0.112		0.264		
		Average		883		0.146	0.136		0.050		0.050		0.123		
Average percentage of SO <sub>2</sub> absorbed														68%	65%
Average rate of absorption (mg. SO <sub>2</sub> per minute per 1000 gm. of leaves)														1.4	0.33
Total SO <sub>2</sub> absorbed (grams)														30.94	
Total S absorbed (grams)														15.47	

\* The maximum and minimum values in the horizontal lines of this table occurred in different fumigations and are, therefore, unrelated.



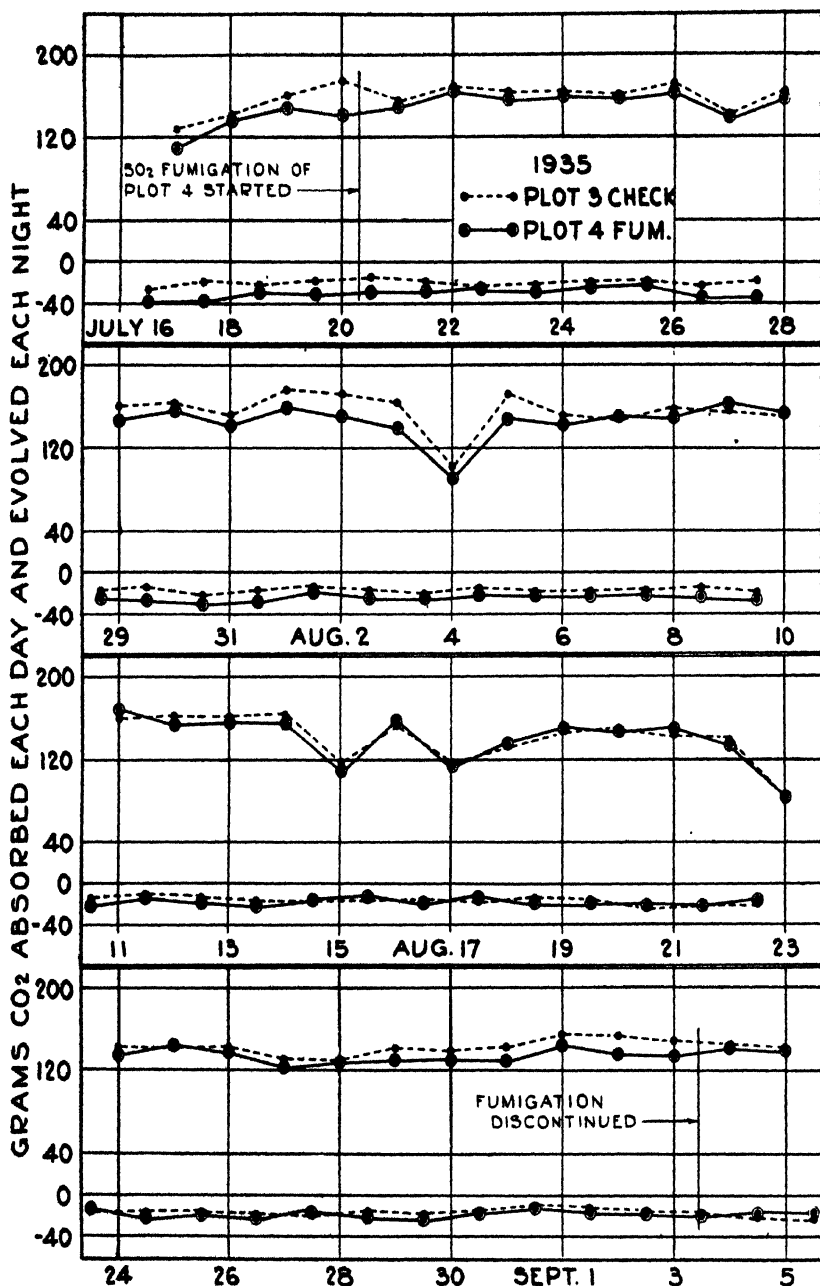


FIG. 11. Total daily apparent assimilation and respiration of plots 3 and 4 at Logan, Utah (1935). Plot 4 was fumigated continuously from July 20 to September 3 (45 days) with 0.14 p.p.m. SO<sub>2</sub>.

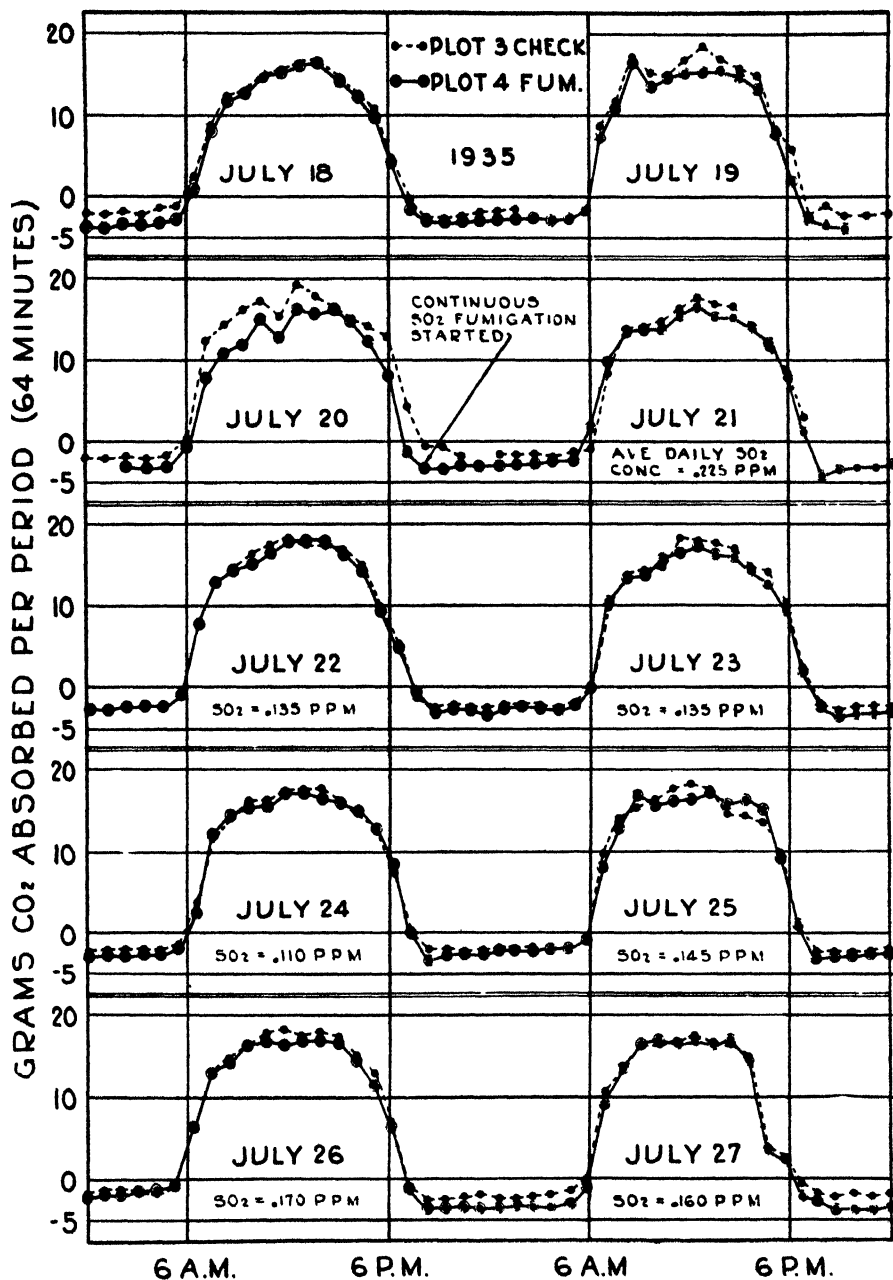


FIG. 12. Chart A, July 18-27, 1935. Apparent assimilation and respiration of plots 3 and 4 at Logan, Utah. Plot 4 was fumigated continuously from July 20 to September 3 (45 days) with 0.14 p.p.m.  $\text{SO}_2$ .

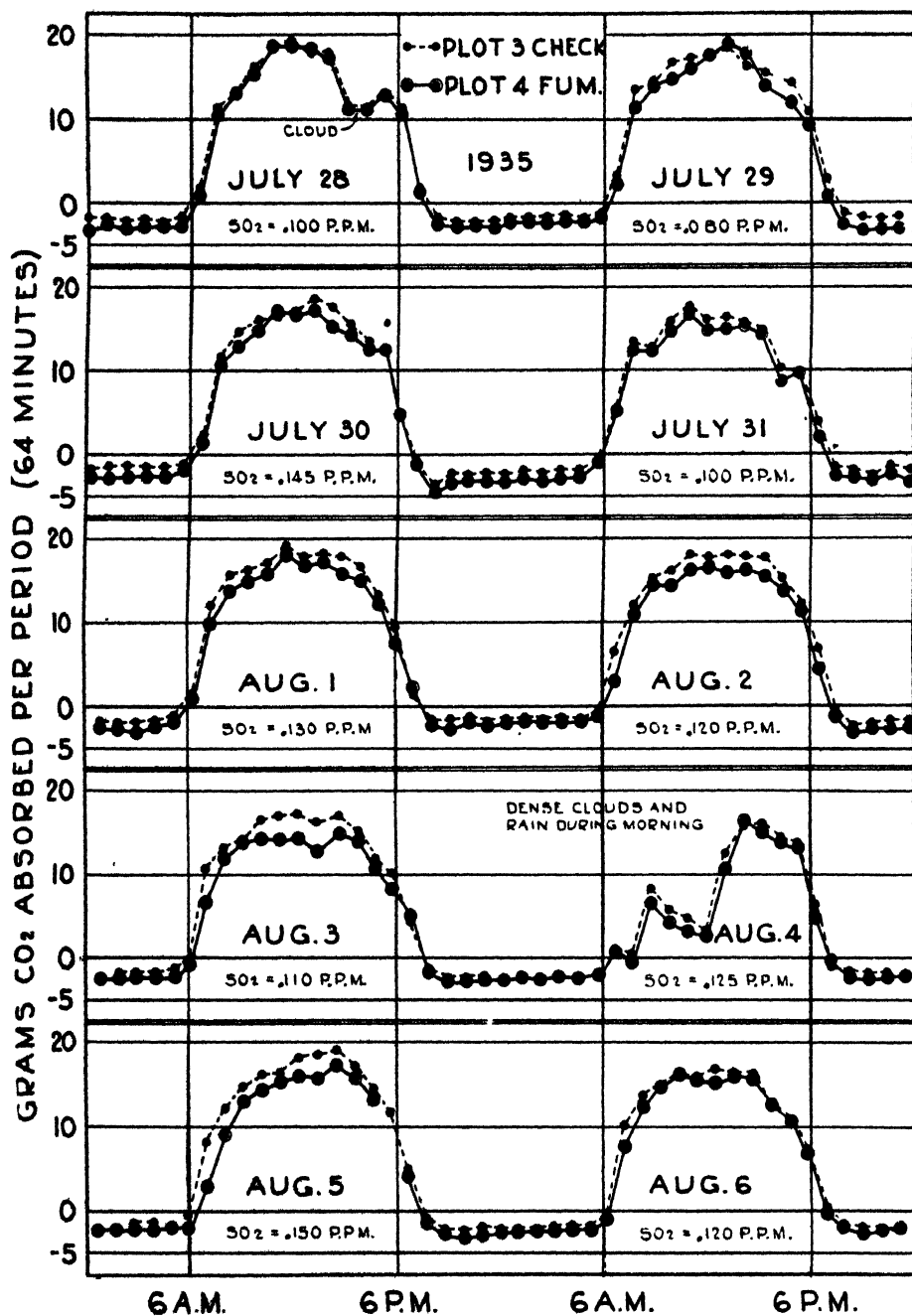


FIG. 12. Chart B, July 28-August 6, 1935.

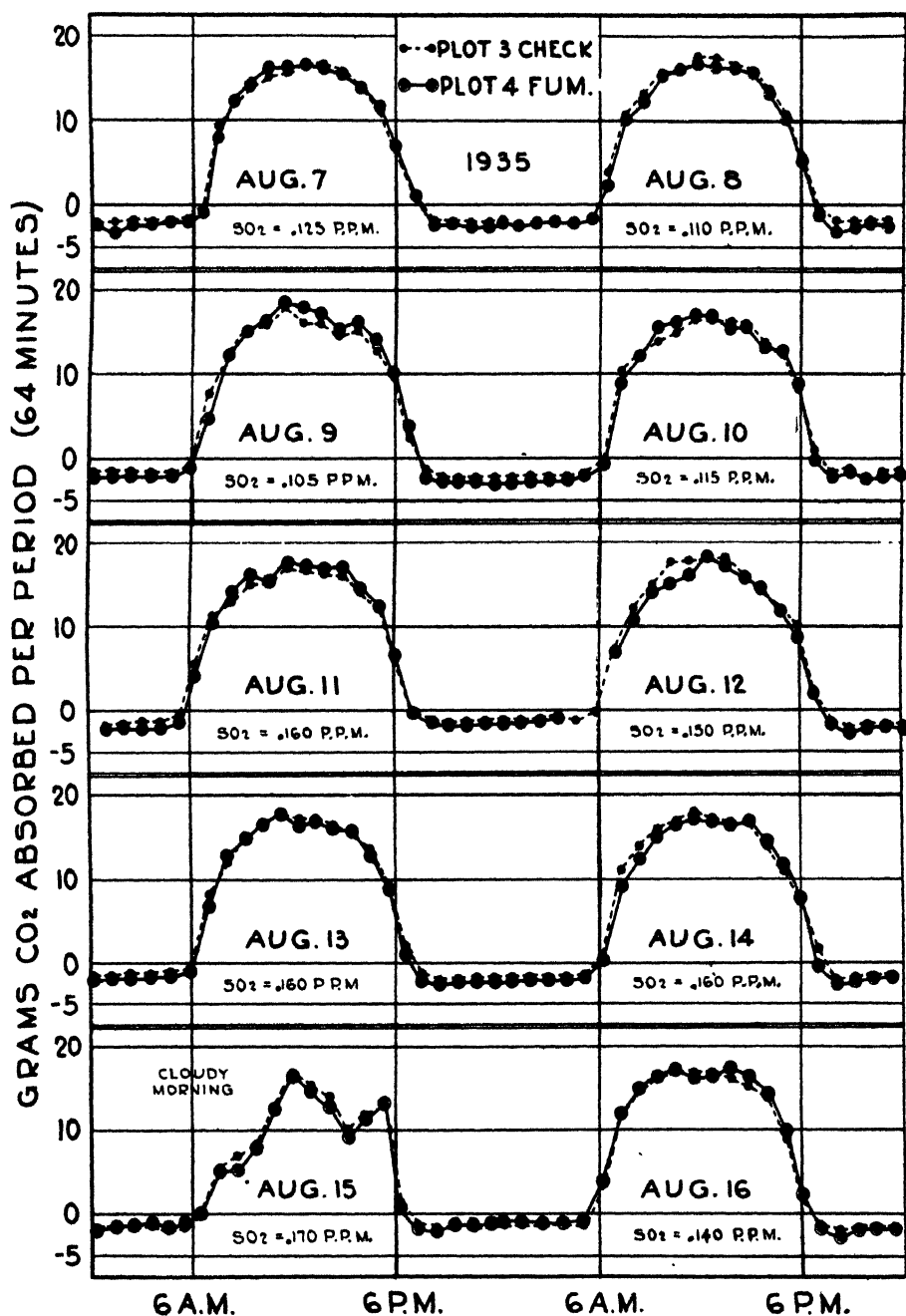


FIG. 12. Chart C, August 7-16, 1935.

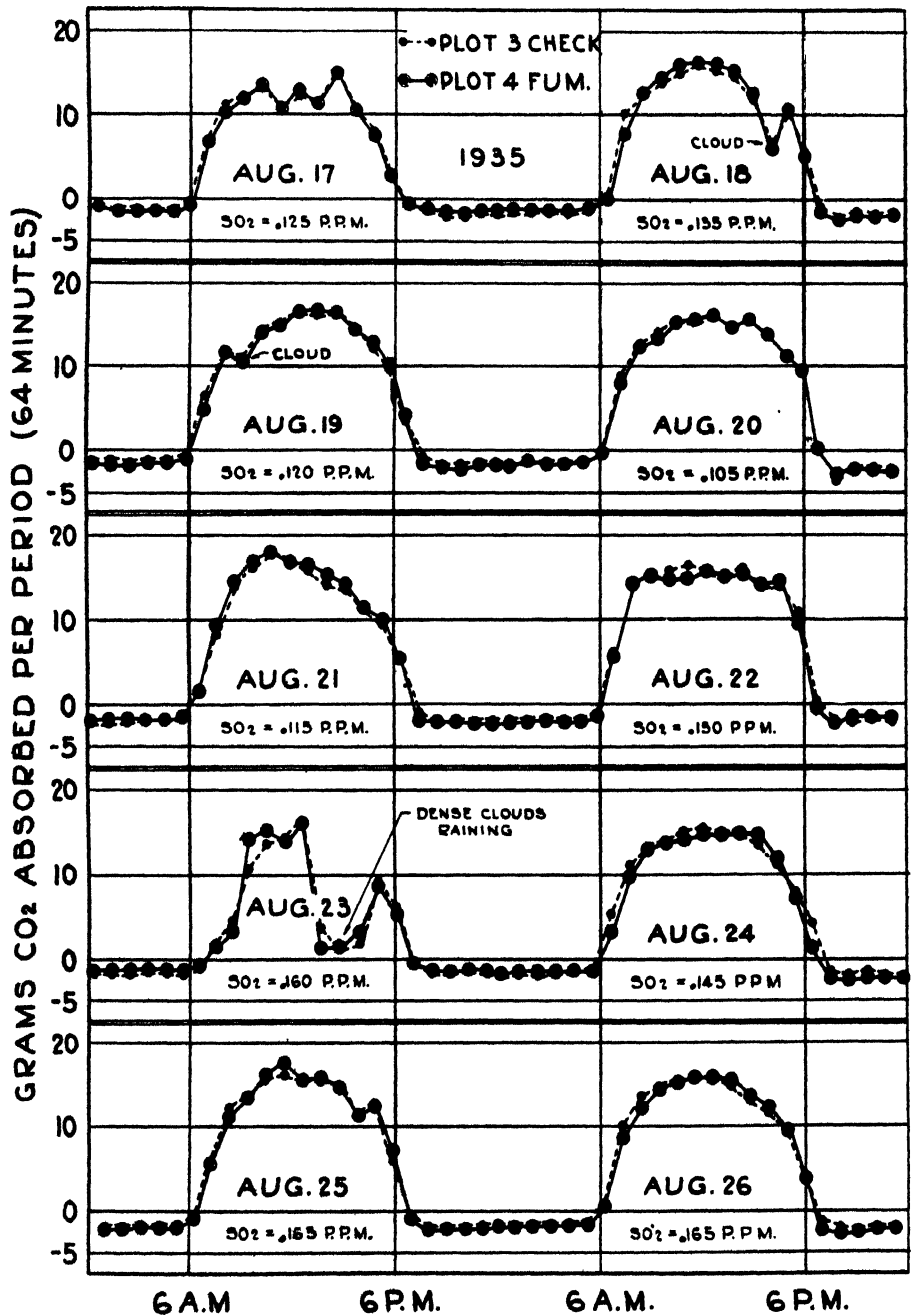


FIG. 12. Chart D, August 17-26, 1935.

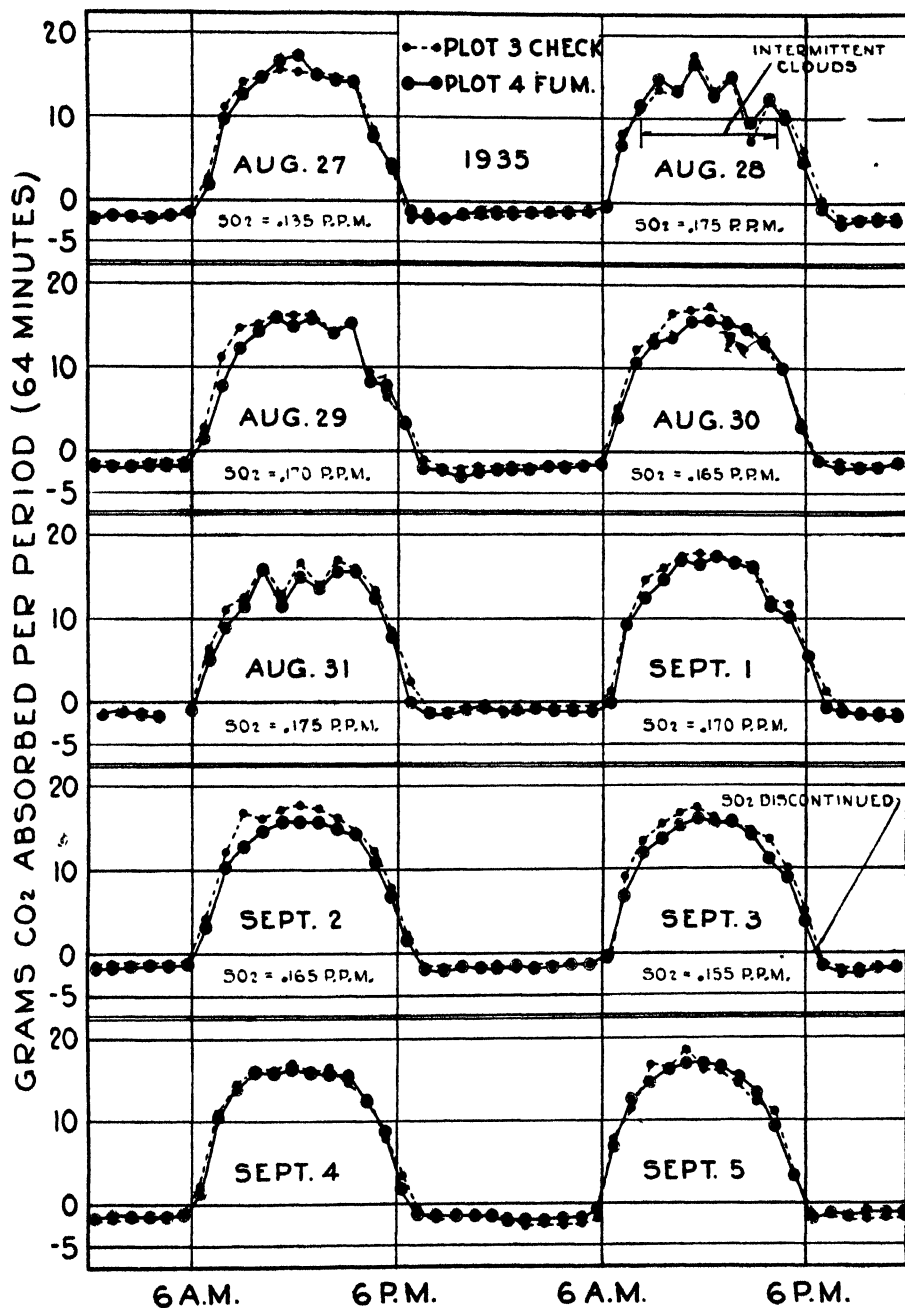


FIG. 12. Chart E, August 27-September 5, 1935.

it had been before the fumigation started. Then for 8 days the rate of apparent assimilation of the treated plot fell off about 7 per cent. owing, we believe, to failure of irrigation water to penetrate the plot. During this period the treated plot seemed to have a yellowish appearance, as noted by several observers, and it was estimated that its color was about 10 per cent. more yellow than the check plot. After a heavy irrigation of the plots on August 3, the color of plot 4 seemed to improve, so that a few days later no differences could be observed. After August 6, the curves of apparent assimilation showed an increase of 2 per cent., and the net assimilation an increase of 6 per cent. and 8 per cent., suggesting a stimulation of photosynthesis by the sulphur dioxide treatment. During the last 6 days of the fumigation, owing possibly to an increased concentration of sulphur dioxide, which averaged about 0.17 p.p.m. during this period, the apparent assimilation of the treated plot fell off 4 per cent., and the net assimilation receded to normal. Finally, after the fumigation was discontinued, the curve of apparent assimilation of the fumigated plot for 2 days before the plots were harvested was 98 per cent. of the check, while the net assimilation was 107 per cent. of the check. It should be noted that the total amount of respiration of plot 4 was 26 per cent. greater than that of plot 3, but the difference decreased as the experiment proceeded. This circumstance favors, perhaps unduly, the treated plot in the adjusted ratios of the net assimilation. This difference of respiration may have been caused by a difference in "soil respiration" of the two plots already referred to.

This detailed record of the carbon dioxide exchange, together with the yield data, would seem to be conclusive evidence that this prolonged fumigation at this low concentration produced no deleterious effect upon the growth of alfalfa, and for about half the time a definite stimulation is suggested. The reduced rate of assimilation which was indicated during the last 6 days of the fumigation represents a decrease of only 1 per cent. of the total assimilation of the treated plot over the period of the experiment.

Sulphur and chlorophyll data are presented in table XXV. The amounts of chlorophyll were nearly identical in plots 3 and 4 after August 6. Unfortunately, preparations had not been made earlier to carry out these analyses when differences possibly may have existed. The sulphur determinations showed a regular increase in the sulphur content of the leaves and stems of plot 4, but the maximum amount found in the leaves was 1.52 per cent., which was insufficient to interfere with the functions of the leaf. Table XXIII indicates that 30.94 gm. of sulphur dioxide were absorbed from the air, equivalent to 15.47 gm. of sulphur. Only 6.937 gm. of this amount was recovered in the plant tissue (table XXV). As was noted above, part of this sulphur appeared on the second 1936 crop, when more sulphur was found in the top growth than was added by the fumigation treatments.

TABLE XXIV

BALANCE SHEET OF CARBON DIOXIDE EXCHANGE AND GROWTH OF ALFALFA PLOTS 3 AND 4—1935

DATE (1935)	TOTAL APPARENT ASSIMILATION CO <sub>2</sub>				NIGHT RESPIRATION CO <sub>2</sub>				NET ASSIMILATION CO <sub>2</sub>					
	PLOT 3 CHECK	PLOT 4 FUMI- GATED	RATIO 4/3		PLOT 3 CHECK	PLOT 4 FUMI- GATED	RATIO 4/3		PLOT 3 CHECK	PLOT 4 FUMI- GATED	RATIO 4/3			
			ACTUAL	ADJUSTED			ACTUAL	ADJUSTED			ACTUAL	ADJUSTED		
	gm.	gm.	%	%	gm.	gm.	%	%	gm.	gm.	%	%		
7/17- 7/20	609	541	89	92	Before fumigation									
7/18- 7/19	304	287	95	98	69	121	175	126	540	420	78	86		
					36	56	156	112	268	231	86	95		
	Continuous fumigation started at 8:40 P.M. July 20—average SO <sub>2</sub> concentration 0.141 p.p.m.													
7/21- 7/28	1300	1260	97	100	157	218	139	100	1143	1042	91	100		
7/29- 8/ 6	1415	1271	90	93	157	225	143	103	1258	1046	83	91		
8/ 7- 8/16	1525	1507	99	102	159	198	125	90	1366	1309	96	106		
8/17- 8/26	1337	1325	99	102	179	186	104	75	1158	1139	98	108		
8/27- 9/ 2	993	929	93	96	107	123	115	83	886	806	91	100		
	Fumigation discontinued at 6:45 P.M., September 3													
9/ 3- 9/ 5	439	419	95	98	61	51	84	61	378	368	97	107		
Totals	7618	7252	95	98	889	1122	126	91	6729	6130	91	100		
	Equivalent dry matter (44 per cent. carbon)				(grams)				4171 3799					
	Functioning tissue				Plot 3				Plot 4					
	Interim samples for analysis				105 <sup>a</sup>				103 <sup>a</sup>					
					199 <sup>b</sup>				200 <sup>b</sup>					
	Harvest September 6				483 <sup>a</sup>				460 <sup>a</sup>					
					860 <sup>b</sup>				963 <sup>b</sup>					
					135				227					
	Dead tissue September 6													
	Total tissue to September 6								1782 1953					
	Dry matter July 17 (estimated from comparable alfalfa harvested on July 17) (grams)								737 762					
	Top growth during experiment (grams)								1045 1191					
	Top growth—percentage of total CO <sub>2</sub> assimilated								25.0 31.4					
	Probable root increment (grams)								3126 2608					



TABLE XXV  
ANALYSES OF PLOTS 3 AND 4 FOR SULPHUR AND CHLOROPHYLL—1935

DATE (1935)	PLANT TISSUE	SULPHUR (S)				CHLOROPHYLL				
		PLOT 3 (CHECK)		PLOT 4 (FUMIGATED)		PLOT 3 CHECK	PLOT 4 FUMI- GATED	OUT- SIDE* CHECK		
		WEIGHT OF TISSUE	SULPHUR (S)	WEIGHT (S)	WEIGHT OF TISSUE				SULPHUR (S)	WEIGHT (S)
July 18	Leaves	gm.	%	gm.	%	gm.	%	%		
" 27	"	4.6	0.432	0.020	8.5	0.429	0.036	.....		
August 2	"	13.5	0.464	0.063	15.3	0.952	0.146	.....		
" 6	"	21.3	0.448	0.095	19.6	1.027	0.201	.....		
" 16	"	18.6	0.438	0.081	17.0	1.240	0.211	.....		
September 6	"	32.7	0.441	0.144	30.0	1.475	0.443	.....		
" 6	"	483	0.480	2.318	460	1.520	6.992	.....		
" 6	Dead leaves	44	0.380	0.167	51	0.882	0.450	.....		
July 18	Stems	6.5	0.163	0.011	11.1	0.151	0.017	.....		
" 27	"	24.1	0.130	0.031	28.3	0.140	0.040	.....		
August 2	"	40.2	0.136	0.055	37.9	0.163	0.062	.....		
" 6	"	35.1	0.171	0.060	34.7	0.177	0.061	.....		
" 16	"	64.4	0.135	0.087	60.7	0.225	0.137	.....		
September 6	"	860	0.166	1.428	963	0.257	2.475	.....		
" 6	Dead stems	100	0.160 <sup>a</sup>	0.160	193	0.200 <sup>a</sup>	0.386	.....		
" 6	Leaves and stems	.....	0.278	.....	.....	0.665	.....	.....		
Total		4.720				11.657				
Excess of S in plot 4 over plot 3		.....				6.937				

\* Comparable alfalfa grown adjacent to and outside of plant chambers.

<sup>a</sup> Estimated.

Evidently there was some translocation of the sulphur to the roots in this 1935 experiment.

Finally, it may be pointed out that the "rate of absorption" of sulphur dioxide in this series of experiments, as shown in the tables of fumigation data, is a valuable index to the effectiveness of the treatments in affecting photosynthesis. There is a gradual lowering of this rate of sulphur dioxide absorption in the series, until in the last experiment, the gas could be oxidized as rapidly as it was absorbed, so that it had no effect in reducing photosynthesis over a very protracted period.

### Discussion

The experiments described in this paper were undertaken primarily to find out if concentrations of sulphur dioxide which did not produce any visible injury to the plant, whether applied as high concentrations of short duration, as low intermittent concentrations, or as continuous fumigations throughout the life of the plant, nevertheless could cause injury to the protoplasm or to any plant structure. In other words, it was the purpose to find out whether "invisible injury" really existed and, if it did exist, how far-reaching an effect it could have.

The experiments have shown definitely that sulphur dioxide can produce reductions—the amount depending on the intensity of the fumigation—in the rate of photosynthesis during the actual duration of the fumigations, and sometimes for a number of hours thereafter, particularly in cases in which considerable sulphur dioxide previously has been absorbed, without producing any visible effect on the leaves. As soon as the fumigations were over, however, the rate of photosynthesis returned more or less quickly to normal. The effect upon photosynthesis is not dissimilar to that produced by a passing cloud, except that the cloud shadow usually produces an effect several times larger. The return to normal is frequently as rapid as the return after a cloud passes. Though there is a reduction in rate in both cases, the complete return afterward is definite indication that no injury has occurred in either case. Since the rate of photosynthesis is so delicate a yardstick, capable of registering in a definite way even the slightest effect upon any of the functions of the plant, it is hard to conceive of any injury which would not be clearly reflected by a more or less permanent change in the photosynthetic level.

Though the attempt has been made many times, and in many ways, to apply sulphur dioxide to plants in such a manner as to reduce the yield of the crop without producing visible injury, this has not been accomplished in any of our experiments. Further confirmation for wheat was shown by SWAIN and JOHNSON (4), who found no interference with reproduction processes and no reduction in the yield of that grain, as a result of fumiga-

tions with sulphur dioxide for several hours daily in concentrations too low to cause visible markings.

Many toxic substances are known to produce a stimulation in plant activity when applied in subtoxic quantities. Sulphur dioxide seems to be no exception to this rule. In addition, sulphur dioxide is rapidly oxidized to the sulphate form by the plant, which is the form in which plants use sulphur in their nutrition.

Just what percentage of sulphur in plant tissues is optimum for plant growth has not been determined. These experiments have shown that it can vary through a wide range, certainly from 0.5 per cent. to 1.5 per cent. in alfalfa leaves, without affecting the photosynthetic level. As 2 per cent. sulphur is approached, alfalfa leaves begin to show chlorotic markings, which rapidly increase as the percentage of sulphur increases.

### Summary

1. Nine fumigation experiments with alfalfa plots and their corresponding checks are described, in which the treatments range from fumigations which produced almost complete foliar destruction, to long-continued sublethal dosages of the gas. The effects of the treatments have been determined by measuring the rate of photosynthesis and respiration of the plot in comparison with a similar check plot extending for a period from several days before the fumigations were started to several days after they were discontinued. Yield data confirm the carbon dioxide exchange data. The observations on the effects of the fumigations on the plants have been supplemented by chemical analyses of the leaves and stems for sulphur and chlorophyll.

2. Extensive foliar destruction of alfalfa by a sulphur dioxide fumigation is followed by a rather rapid growth of new leaves and a corresponding re-establishment of photosynthetic activity. In one experiment the latter was restored to about 75 per cent. of normal in 9 days following a fumigation which caused 90 to 95 per cent. of leaf destruction. In another similar experiment 65 per cent. recovery of photosynthesis occurred in 10 days, and 80 per cent. in 15 days.

3. Short fumigations with high concentrations of sulphur dioxide which were discontinued before an appreciable amount of leaf destruction was produced, caused large reductions in the rate of photosynthesis during the fumigation, but the photosynthesis began to increase within an hour after the treatment. The effect of such fumigations could be observed in a somewhat lowered rate of apparent assimilation for about 2 days after the treatment. Thereafter the rate was normal or greater than normal. The effect of two such treatments on the yield of a single crop of alfalfa, as calculated from the carbon dioxide exchange data, agrees with the decrease in yield calculated from a yield-leaf destruction equation previously published.

4. A number of short fumigations, with concentrations ranging from 0.7 to 1.26 p.p.m., each caused a definite decrease in the photosynthetic level; but immediately thereafter the activity rose to a normal or greater than normal level, so that when the total assimilation was calculated the decreased assimilation that had occurred during the fumigation was largely counterbalanced by increased assimilation after the fumigation was discontinued. The net effect of the fumigations was, therefore, practically zero. The results suggest that subsequent to such fumigations there was a slight stimulation of assimilation.

5. A plot was fumigated for 4 hours on each of 3 successive mornings with about 0.6 p.p.m. After several days this treatment was repeated. During the time each fumigation was in progress the assimilation level fell to about 75 per cent. of the check, but returned practically to normal in the afternoon. The 6 fumigations caused a decrease in net assimilation of about 10 per cent. of the total assimilation during the 16 days of observation or about 5 per cent. of the net assimilation of a full-crop period.

6. Another plot was fumigated on 5 successive mornings with about 0.43 p.p.m. Then after 5 days this treatment was repeated. During each of these fumigations the average level of apparent assimilation was reduced to 88 per cent. of the check, but the level returned to a normal or greater than normal level as soon as the fumigation was discontinued. Again, the reduced rate of apparent assimilation during the fumigation periods was largely counterbalanced by increased assimilation subsequent to the fumigations, so that a significant reduction in the total amount of assimilation during the experiment probably did not occur.

7. A plot was fumigated on 31 days, 27 of them in the morning, with 0.35 p.p.m. for 4.25 hours each day. In addition to the fumigation treatments, water was withheld from the plots for 3 weeks so that at the end of this period temporary wilting occurred on both plots during the hot part of 3 days. The average effect of these fumigations was to reduce the assimilation level about 6 per cent. during the progress of the fumigation, but immediately after the fumigation was discontinued the assimilation level practically returned to normal. The drought did not modify the effect of the fumigation, as shown by the relative photosynthetic activity of the two plots in the morning and afternoon hours, even on the three days when wilting occurred.

8. Three long continuous fumigations in 1935 showed that no effect of the fumigation occurred with 0.24 p.p.m. for about 3 days, with 0.19 p.p.m. for 11 days, and with 0.14 p.p.m. for at least 39 days. The data even suggest a slight stimulation of apparent assimilation during these periods. The continued application of the higher concentrations caused a gradual reduction in the photosynthetic level of the treated plot, with attendant visible

markings of the leaves and premature shedding of the older leaves. The reduced rates of photosynthesis were paralleled by reduced amounts of functioning leaf tissue.

9. Chlorophyll analyses in these experiments showed a definite tendency for the longer fumigations to reduce slightly the chlorophyll content in comparison with that of the check plot, but comparable alfalfa growing outside of the plant chambers usually had less chlorophyll than the treated plot. The reduction in the chlorophyll content of the treated plots was probably without practical significance in every case, except the 2 long-continued 1935 fumigations with the higher concentrations of sulphur dioxide, which produced appreciable visible markings. The chlorophyll content of the plot which received 0.14 p.p.m. continuously was normal.

10. The sulphur analyses of the leaves and stems account for about 90 per cent. of the sulphur dioxide added in the short fumigations. The remainder was probably absorbed on the walls of the plant chamber and in the soil. The continuous fumigations of 1935 exhibited a large deficit of sulphur in the plant tissue as compared with the sulphur dioxide added. It was shown that most of this deficit in one experiment was transferred to the roots and could be accounted for in the crops of 1936.

11. Absorption by the soil was too slight to be determined analytically. Fourteen times as much sulphate was found in the soil adhering to the roots as in the soil mass, suggesting a passage outward of sulphate from the roots to the soil.

12. The rate of absorption of the gas by the leaves decreased in this series of fumigations along with decreasing effects on photosynthesis of the fumigation treatments.

13. Leaf analyses showed large increments of sulphur in the protracted fumigations. When the sulphur content of the leaf approached 2 per cent., the leaf became chlorotic and soon ceased to function. At about 1.5 per cent. to 1.7 per cent. or less, the leaf appeared to be normal.

14. The complete return to its normal level of photosynthesis, following fumigations which did not produce visible injury, would indicate that in such fumigations there is no "invisible injury," either to the protoplasm or other plant structure.

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# INFLUENCE OF WEAK ELECTRIC CURRENTS UPON THE GROWTH OF THE COLEOPTILE

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(WITH THREE FIGURES)

## Introduction

Among the numerous contributions to the electrophysiology of the plant there were until lately no investigations which dealt with the problem of the relation between bioelectrical phenomena and growth, the main reason being the absence of a satisfactory theory. The purely empirical data, which have been obtained by various authors, could not attract the attention of physiologists to this problem. Interest in electrophysiological investigations of growth has increased lately, since the studies of the growth hormone have thrown a new light upon this phenomenon. The first stimulus in this direction was given as a result of the ideas developed by one of the writers (3) on the effect of electrophysiological polarization upon the translocation of the growth hormone in organs of higher plants. When one compares the data of BOSE, BRAUNER, WALLER, and other physiologists on the effect of geo- and photoinduction on changes in the electric potentials of various parts of the plant organism, it is easy to notice that growth is always taking place in such a way that the concentration of the growth hormone increases in the positively charged regions and diminishes in those regions where, according to electrometric observations, the plant has a negative charge. On the basis of this comparison the conclusion can be drawn that in organs, which are subjected to the influence of photo- and geinduction, the growth hormone is transferred in the direction of the increasing potential.

In further development of this idea WENT (12) has made the assumption that, in general, the translocation of the growth hormone, in organs of the plant from places where it is produced to the growing zone and beyond, depends on the distribution of the electrical potential. WENT showed that the basic dyes diffuse in sections of the stalk of *Impatiens balsamina* in the direction of the apex, while the acid dyes diffuse toward the basal part, which, according to WENT, always has a positive charge. Auxin, as determined by KÖGL (8), is an acid. Therefore WENT considers that the anion of this acid, which is its physiologically active part, must move in the stalk in the same direction as the acid dyes do, i.e., from the apex to the base. However, one does not find in WENT's work any experiments which would directly confirm this assumption about the translocation of the growth hormone.



In 1933, KÖGL (8) described experiments which were based on the observation that in various hours of the day the decapitated coleoptiles of oats react to the same dose of auxin by curvatures of different intensity (when the experiments were carried out according to the method of WENT, 11). Searching for the cause of this phenomenon KÖGL came to the idea that the effect of atmospheric electricity, namely, the ionization of the air, might be the cause of these differences. This idea seems to be confirmed by the fact that when coleoptiles are placed in zinc or tin containers they show a considerably greater "sensitivity" and the fluctuations in the intensity of the curvatures during the day are far less than in cases where the experiments are carried out in the ordinary way. On the assumption that the cause of the observed changes in sensitivity of the coleoptiles is the passing over the plant of an electric current of very low intensity, KÖGL carried out the following experiments. A very weak electric current of approximately 0.0008 milliampere was passed through decapitated coleoptiles of oats, to one side of which there was attached a small cube of agar-agar containing auxin. One of the electrodes—a moistened silk thread—was attached to the agar cube, while the other electrode was connected with the basal portion of the coleoptile. The results of this experiment showed that when the agar cube was connected with the negative pole, the coleoptiles reacted to the same quantity of growth hormone by more vigorous bending than when the cube was connected with the positive pole. The ratio between the intensities of the curvatures was 12 : 1. KÖGL explained these results by WENT's scheme, assuming that in case of the first contact "the transport of the physiologically active auxin anions to the base of the coleoptile is accelerated," while in the other case it is retarded.

In 1934 KOCH (7), of GUTTENBERG's laboratory, published a paper, in which a whole chapter is devoted to the problem of translocation of the growth hormone in coleoptiles of oats and also in the hypocotyls and roots of sunflower and some other plants placed in the electric field, when an electric current passed through these organs. The starting point of the first group of experiments of this author were the investigations of BRAUNER and BÜNNING (2), which showed that the coleoptiles of oats bend toward the negative pole when placed in an electric field of high intensity, between two metallic plates attached to the poles of a battery. On the other hand, the root of the broad bean (*Vicia faba*) under the same conditions bends in the opposite direction. The authors explain this phenomenon as being due to the deviation of the growth hormone flow in the direction of that part of the organ, which became positively charged due to induction. HARTMANN (6) repeated these experiments, but obtained different results. On the basis of this work the author came to the conclusion that the influence of the electric field upon the growth of the plant is a more complicated

phenomenon and that it cannot be explained by the translocation of the growth hormone to the positively charged side of the organ.

KOCH attempted to clear up this contradiction, but unfortunately he carried out his experiments in such a way that they cannot be compared with the BRAUNER-BÜNNING and the HARTMANN experiments. Instead of placing the plant between the electrodes in atmospheric air, as previous authors did, he immersed them in water into which there were also immersed the electrodes in the form of platinum plates attached to the poles of a flashlight battery, which gave a current of 0.2 ampere at a tension of 4.4 volts. It is clear that under such conditions KOCH's "electric field" created not an induction of + and - charges on the opposite ends of the plant organism, but the passage through it of a current of unknown strength, but which was less than 0.2 ampere. KOCH assumes that the phenomenon of induction should take place in the coleoptile of oats even when it is immersed in water, since the cuticle of this organ, according to the author, acts as an insulator. The experiments, however, show that the cuticle of the coleoptile as well as of other plant organs, especially in the wet state, exercises a very small resistance to the electric current. Therefore, under the conditions of KOCH's experiments, the current was passing in the plant in the same direction as in the surrounding water.

Of much greater interest are KOCH's experiments in which he is trying to determine whether the growth hormone of oats, under the influence of an electric current, really moves in agar-agar to the positive pole, as it should, according to WENT's theory of polarization. KOCH placed a certain quantity of coleoptile tips on an agar plate which was 1-1.5 cm. in length and 0.5 cm. in width. At the sides of this plate he placed platinum electrodes and passed a current from a flashlight battery (4.4 volts). Then the author cut out cubes from various parts of the plate, put them unilaterally upon the decapitated coleoptiles of oats and after a definite period measured the curvatures of the coleoptiles. The greatest curvatures were formed when cubes which were cut out near the positive pole were used. Cubes taken from the opposite part of the agar plate (at the negative pole) did not cause any bending. On the basis of these results the author concludes that under the influence of the electric current the growth substance moves in agar toward the positive pole.

It is easy to show, however, that these experiments are not definite. When an electric current passes through an agar plate the phenomenon of electrolysis is undoubtedly taking place in it and this causes the accumulation of the products of hydrolysis at the electrodes in the agar plate. Basic products of electrolysis at the negative pole, uniting with the growth hormone which is an acid, will transform it into an inactive state of a salt. It is, therefore, not known to what the results of KOCH's experiments have to

be ascribed: whether to the translocation of the hormone to the positive electrode, to its inactivation at the negative electrode, or to the combined activity of these two factors.

The third series of KOCH's experiments likewise is not very convincing. These were carried out on young hypocotyls of *Helianthus* and *Lupinus*. The author inserted the positive electrode into the side of the upper part of the hypocotyl to a depth of about 1 mm.; the negative electrode was inserted into the opposite side of the hypocotyl, 2 cm. lower than the positive one. An electric current from a flashlight battery was passed through the organ for 30 minutes. Two hours later definite curvatures had formed in the direction away from the positive pole. This phenomenon, according to KOCH, shows that the growth substance is attracted by the positive pole and thus an acceleration of growth on this side takes place. This conclusion, however, cannot be considered as established either. The cause of the acceleration of growth might have been a simple increase in acidity at the positive electrode due to electrolysis. STRUGGER (10), for instance, has shown recently that it is possible to accelerate the growth of cells at the growing zone (hypocotyl of the sunflower) with the aid of acid solutions of a definite pH. His results have been partially confirmed by BONNER (1), who considers that the acceleration of growth, by increasing the acidity, results from an activation of the growth hormone reserves: the inactive salt is changed to the active form of a free acid. Therefore, KOCH's experiments do not seem to prove the translocation of a growth hormone in the hypocotyl tissue to the positive pole.

Finally the work of RAMSHORN, of RUHLAND's laboratory, has to be mentioned. In his electrophysiological investigations this author applied more perfect methods than KOCH and used electrodes which did not polarize. By determining the difference in the electric potentials between various points of the root, stalk, and leaves of young seedlings of the sunflower, oats, bean, and other plants with the aid of a quadrant electrometer, he showed that the curves of the electric potential distribution are very much like the curves of the rate of growth of various zones of these organs. The zones which show a more rapid growth are always electropositive as compared to those which grow slower. Changes in the difference of growth, which are connected with nutations, cause corresponding changes in potentials. Alterations in the difference of potentials may also be created by the introduction of the growth hormone in certain places of the organ. In this case again the zones which grow faster will become electropositive.

RAMSHORN studied also the effect of an outside electromotive force upon the growth of the hypocotyl of the sunflower and found that when the growing zone is connected with the positive pole an acceleration of growth of short duration may be observed. On the other hand, when it is connected

with the negative pole, then growth is retarded. Without going into details of other results of RAMSHORN's work, we will only mention his experiments on sections of the stalk of *Impatiens balsamina*, on which WENT carried out his studies. These experiments showed that the apical part of the stem is electropositive with respect to the basal one. On the basis of these results RAMSHORN came to the conclusion that the unequal diffusion of acid and basic dyes, which WENT observed on this object, cannot be explained by differences in electric potentials.

The brief review of the latest work in the electrophysiology of growth, which has been presented, shows that the conclusions of various workers in this field differ greatly even on purely experimental questions. As was shown in the review, the methods applied by some of the authors mentioned were not always free from criticism from the physical and physiological points of view. However, such preliminary work is necessary. In view of these considerations we believe it useful to publish briefly the main results of our electrophysiological studies, which were planned and partially carried out before the papers of KOCU and of RAMSHORN appeared, and with which our work has some common points.

The starting point of our investigations were KÖGL's experiments mentioned above. If an electrical current of  $10^{-6}$  to  $10^{-7}$  amperes really accelerates or retards, depending on the direction of the current, the translocation of the growth hormone in the decapitated coleoptile, it may be assumed that the same phenomenon should also take place in the undecapitated coleoptile which is obtaining the growth substance from its own top, namely, that the rate of growth would change under the influence of the growth hormone depending upon the direction of the electromotive force which is applied to it. If the WENT-KÖGL scheme is correct, then the current, which is passing from the base to the apex of the coleoptile, should cause a certain acceleration in growth while the current, which is directed to the opposite side, should retard growth. The main object of our experiments was to verify this conclusion.

### Methods

First we will describe the method used. In all our investigations we applied only such electrodes as do not polarize and which were described previously by one of the writers (5). As will be seen from figure 1, such an electrode is made of two communicating glass tubes, *A* and *B*. The wider tube, *A* (about 7 mm. in diameter and 60 mm. long), is filled with a saturated solution of  $\text{ZnSO}_4$ , while the narrow one, *B* (2 mm. in diameter), is filled with tap water. A kaolin stopper, *a*, which is previously soaked in water, and 2 layers of cotton, *b*, separate the water in the capillary tube, *B*, from the zinc sulphate solution in *A*. An amalgamated rod, *d*, with a sold-

ered clamp, *e*, is put into the tube, *A*, through a rubber stopper, *f*. A silk thread, *i*, of which one end is immersed in water, is connected by its other end with the object under investigation.

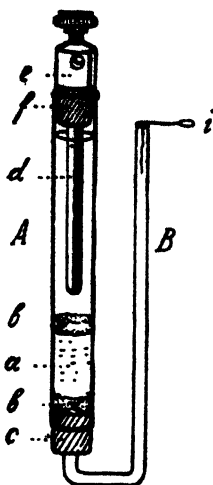


FIG. 1. Electrode used in the work reported.

Figure 2 shows how the two electrodes are fastened to the stand, which is made of a paraffined cork and glass rods (5). This figure also shows how the electrodes are connected with the coleoptile, which was at a certain time before the experiment cut off from the mesocotyl and freed from the primordial leaf (4). The coleoptile is placed by its lower end into a cut of the cork plate, *a*, which is in turn attached by a nickel pin to a paraffin layer in a cylindrical glass container, *c*.

As was mentioned above, both electrodes were connected with the coleoptile through silk threads. One of these threads had on the end a loop of about 0.5 mm. in diameter. This loop was set before the beginning of the experiment on the top of the coleoptile (fig. 2). The other electrode, also through silk thread, was connected with the water, which was on the paraffin layer in the container, *c*, and into which the coleoptile was immersed by its lower end. It must also be mentioned that the electrodes were always arranged in such a way that the open ends of the capillaries, *B*, which are filled with water, were on the same horizontal plane with the point of attachment of the silk threads to the object (fig. 2). Thus we avoided the formation of a siphon and the running over of the water from the tubes of the electrodes to the object, or in the opposite direction over the threads.

The stand with the electrodes and with the plant was covered by a glass bell jar throughout the experiment. A well-moistened sponge was kept under the bell jar. The experiments were carried out in a dark room, the

temperature of which changed but little. The temperature fluctuations during the course of our experiments usually did not exceed  $0.1$  to  $0.2^{\circ}\text{C}$ . Growth was measured by an horizontal microscope with an ocular micrometer, each division of which was equal to  $50\ \mu$ . The measurements and all the other manipulations were made by the light of a red photographic lamp.

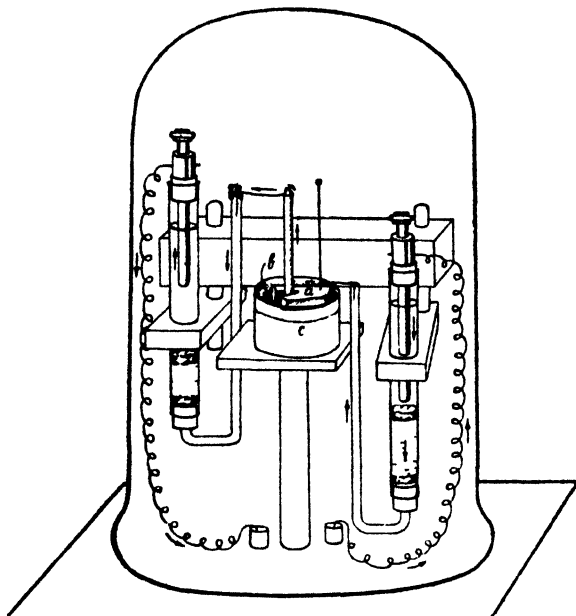


FIG. 2. Electrodes attached to a stand, showing method of making contacts with the coleoptile.

The source of the electric current was an accumulator of 4 volts connected with a rheostat of 120 ohms. The rheostat had a moving contact pointer, which enabled the introduction into the plant of a current of the desired strength through the side connection. The current strength was measured by a galvanometer the sensitivity of which was  $0.14 \cdot 10^{-6}$  amperes. The direction of the current could be reversed by a reversible key. All this arrangement is schematically represented in figure 3.

The main object of our investigation was the isolated coleoptiles of oats (*Avena sativa* var. Siegeshafer Svalöf), which was from 20 to 30 mm. in length. We worked also with the coleoptile of whole uninjured oats seedlings. Several experiments were carried out with rye (*Secale cereale*).

## Experimentation

### ISOLATED COLEOPTILES

The following tables (I-XX) give the results of experiments with isolated coleoptiles. The arrow pointing upward shows that the current ran

from the base of the coleoptile to the apex. The current running in the opposite direction is indicated by arrows pointing downward. Growth is given everywhere in divisions of the scale of the ocular micrometer ( $50\text{ }\mu$ ).

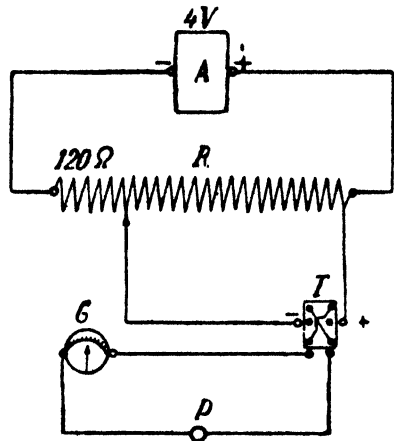


FIG. 3. Diagram of circuits used in making the measurements recorded.

TABLE I

EXPERIMENT, MAY 8, 1934. COLEOPTILE OF *AVENA SATIVA*. MATERIAL PLANTED ON MAY 4. TEMPERATURE 20° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN $50\text{ }\mu$ )
1 .....	amp. 0		1: 20 P.M.	
2 .....	0		1: 40 "	3.5
3 .....	0		2: 00 "	3.5
4 .....	0		2: 20 "	4.5
5 .....	0*		2: 40 "	3.0
6 .....	$1.13 \cdot 10^{-6}$	↑	3: 00 "	5.5
7 .....	"	↑	3: 20 "	4.5
8 .....	"	↑	3: 40 "	3.5
9 .....	"	↑	4: 00 "	3.0
10 .....	"	↑	4: 20 "	1.5
11 .....	"	↑	4: 40 "	1.0

\* Current on.

TABLE II

EXPERIMENT, MAY 9, 1934. COLEOPTILE OF *AVENA SATIVA*. MATERIAL PLANTED ON MAY 5.  
TEMPERATURE 21° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 $\mu$ )
	<i>amp.</i>			
1 .....	0		11: 20 A.M.	
2 .....	0		11: 40 "	3.25
3 .....	0		12: 00 M.	2.75
4 .....	0		12: 20 P.M.	3.25
5 .....	0*		12: 40 "	3.75
6 .....	$1.88 \cdot 10^{-6}$	↑	1: 00 "	5.0
7 .....	"	↑	1: 20 "	4.25
8 .....	"	↑	1: 40 "	3.25
9 .....	"	↑	2: 00 "	2.5
10 .....	"	↑	2: 20 "	2.0

\* Current on.

TABLE III

EXPERIMENT, MAY 21, 1934. COLEOPTILE OF *AVENA SATIVA*. MATERIAL PLANTED ON MAY 17.  
TEMPERATURE 22° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 $\mu$ )
	<i>amp.</i>			
1 .....	0		7: 20 A.M.	
2 .....	0		7: 40 "	3.5
3 .....	0		8: 00 "	3.5
4 .....	0*		8: 20 "	3.5
5 .....	$0.5 \cdot 10^{-6}$	↑	8: 40 "	4.5
6 .....	"	↑	9: 00 "	4.25
7 .....	"	↑	9: 20 "	3.25
8 .....	"	↑	9: 40 "	3.0

\* Current on.



TABLE IV

EXPERIMENT, DECEMBER 5, 1934. COLEOPTILE OF *AVENA SATIVA*. MATERIAL PLANTED ON NOVEMBER 29. TEMPERATURE 14.5° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 $\mu$ )
	<i>amp.</i>			
1 .....	0		2:40 P.M.	
2 .....	0		3:00 "	3.0
3 .....	0		3:20 "	3.0
4 .....	0		3:40 "	3.0
5 .....	0*		4:00 "	3.0
6 .....	$0.5 \cdot 10^{-6}$	↑	4:20 "	4.0
7 .....	"	↑	4:40 "	3.0
8 .....	"	↑	5:00 "	3.0
9 .....	"	↑	5:20 "	2.5

\* Current on.

TABLE V

EXPERIMENT, FEBRUARY 7, 1935. COLEOPTILE OF *AVENA SATIVA*. MATERIAL PLANTED ON FEBRUARY 1. LENGTH OF COLEOPTILE 23 MM. TEMPERATURE 16° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 $\mu$ )
	<i>amp.</i>			
1 .....	0		1:40 P.M.	
2 .....	0		2:00 "	1.0
3 .....	0		2:20 "	1.5
4 .....	0		2:40 "	2.5
5 .....	0*		3:00 "	2.5
6 .....	$0.4 \cdot 10^{-6}$	↑	3:20 "	4.0
7 .....	"	↑	3:40 "	3.25
8 .....	"	↑	4:00 "	2.75
9 .....	"	↑	4:20 "	2.0

\* Current on.

TABLE VI

EXPERIMENT, DECEMBER 19, 1934. COLEOPTILE OF *AVINA SATIVA*. MATERIAL PLANTED ON DECEMBER 13. TEMPERATURE 16° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 $\mu$ )
	<i>amp.</i>			
1	0		6: 20 P.M.	
2	0		6: 40 "	1.5
3	0		7: 00 "	1.0
4	0*		7: 20 "	1.0
5	$0.3 \cdot 10^{-6}$	↑	7: 40 "	<b>2.5</b>
6	"	↑↑	8: 00 "	<b>3.5</b>
7	"	↑↑	8: 20 "	2.5
8	"	↑	8: 40 "	1.5

\* Current on.

TABLE VII

EXPERIMENT, DECEMBER 27, 1934. COLEOPTILE OF *AVINA SATIVA*. MATERIAL PLANTED ON DECEMBER 21. LENGTH OF COLEOPTILE 23 MM. TEMPERATURE 16° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 $\mu$ )
	<i>amp.</i>			
1	0		5: 40 P.M.	
2	0		6: 00 "	1.0
3	0		6: 20 "	1.0
4	0*		6: 40 "	1.0
5	$0.3 \cdot 10^{-6}$	↑	7: 00 "	2.0
6	"	↑↑	7: 20 "	<b>2.75</b>
7	"	↑↑	7: 40 "	1.75
8	"	↑	8: 00 "	1.5

\* Current on.

TABLE VIII

EXPERIMENT, DECEMBER 15, 1934. COLEOPTILE OF *AVENA SATIVA*. MATERIAL PLANTED ON DECEMBER 9. LENGTH OF COLEOPTILE 25 MM. TEMPERATURE 16.5° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 $\mu$ )
	<i>amp.</i>			
1 .....	0		6: 40 P.M.	
2 .....	0		7: 00 "	3.0
3 .....	0		7: 20 "	2.0
4 .....	0		7: 40 "	1.5
5 .....	0*		8: 00 "	1.5
6 .....	$0.3 \cdot 10^{-6}$	↑	8: 20 "	4.0
7 .....	"	↑	8: 40 "	4.0
8 .....	"	↑	9: 00 "	4.5
9 .....	"	↑	9: 20 "	4.5

\* Current on.

TABLE IX

EXPERIMENT, JUNE 22, 1934. COLEOPTILE OF *AVENA SATIVA*. MATERIAL PLANTED ON JUNE 17. LENGTH OF COLEOPTILE 24 MM. TEMPERATURE 20° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 $\mu$ )
	<i>amp.</i>			
1 .....	0		12: 40 P.M.	
2 .....	0		1: 00 "	3.0
3 .....	0		1: 20 "	4.5
4 .....	0		1: 40 "	4.0
5 .....	0*		2: 00 "	4.0
6 .....	$0.3 \cdot 10^{-6}$	↑	2: 20 "	6.5
7 .....	"	↑	2: 40 "	5.0†
8 .....	$0.6 \cdot 10^{-6}$	↑	3: 00 "	7.5
9 .....	"	↑	3: 20 "	4.5

\* Current on.

† Current strength was increased.

TABLE X

EXPERIMENT, JUNE 23, 1934. COLEOPTILE OF *AVENA SATIVA*. MATERIAL PLANTED ON JUNE 19.  
LENGTH OF COLEOPTILE 25 MM. TEMPERATURE 21° C.

OBSERVATION NUMBER	CURRENT STRENGTH	TIME OF OBSERVATION	DIRECTION OF CURRENT	INCREASE IN GROWTH IN 20 MIN. (IN 50 $\mu$ )
	<i>amp.</i>			
1 .....	0		12: 20 P.M.	
2 .....	0		12: 40 "	3.0
3 .....	0		1: 00 "	3.0
4 .....	0		1: 20 "	4.5
5 .....	0		1: 40 "	4.5
6 .....	0*		2: 00 "	4.0
7 .....	$0.3 \cdot 10^{-6}$	↑	2: 20 "	6.0
8 .....	"	↑	2: 40 "	4.5†
9 .....	$0.6 \cdot 10^{-6}$	↑	3: 00 "	5.25
10 .....	"	↑	3: 20 "	5.25

\* Current on.

† Current strength was increased.

The first 10 experiments (tables I to X), which were selected as typical from a great number of other similar ones, show that when a current of the order of  $10^{-7}$  to  $10^{-6}$  amperes is passing from the base to the apex through an isolated coleoptile of oats, a noticeable acceleration of growth is observed. This acceleration continues for quite a long time at relatively low temperatures (about 16° C.). At higher temperatures (about 20 to 22° C.), how-

TABLE XI

EXPERIMENT, MAY 13, 1934. COLEOPTILE OF *AVENA SATIVA*. MATERIAL PLANTED ON MAY 10.  
TEMPERATURE 21° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 $\mu$ )
	<i>amp.</i>			
1 .....	0		1: 20 P.M.	
2 .....	0		1: 40 "	2.25
3 .....	0		2: 00 "	2.75
4 .....	0		2: 20 "	4.0
5 .....	0		2: 40 "	3.5
6 .....	0*		3: 00 "	5.0
7 .....	$0.75 \cdot 10^{-6}$	↓	3: 20 "	4.0
8 .....	"	↓	3: 40 "	3.0
9 .....	"	↓	4: 00 "	3.0
10 .....	"	↓	4: 20 "	2.5

} Average 4.2

} Average 2.8

\* Current on.

ever, it is of a short duration and is sometimes accompanied by a slight decrease in the growth rate. Certain experiments (tables IX, X) show that an increase in the initial current strength during the experiment causes a new rise in the growth curve. This new rise is sometimes even greater than the initial one.

Let us now see how a current passing in the opposite direction, from the top to the base, affects growth of an isolated coleoptile.

TABLE XII

EXPERIMENT, JUNE 9, 1934. COLEOPTILE OF *AVENA SATIVA*. MATERIAL PLANTED ON JUNE 5.  
LENGTH OF COLEOPTILE 27.5 MM. TEMPERATURE 20° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 $\mu$ )
	<i>amp.</i>			
1 .....	0		7: 00 A.M.	
2 .....	0		7: 20 "	3.0
3 .....	0		7: 40 "	2.75
4 .....	0		8: 00 "	3.25
5 .....	0		8: 20 "	3.0
6 .....	0		8: 40 "	3.25
7 .....	0*		9: 00 "	3.0
8 .....	$0.75 \cdot 10^{-6}$	↓	9: 20 "	3.0
9 .....	"	↓	9: 40 "	2.0
10 .....	"	↓	10: 00 "	2.0
11 .....	"	↓	10: 20 "	2.0

Average 3.1

Average 2.0

\* Current on.

TABLE XIII

EXPERIMENT, DECEMBER 10, 1934. COLEOPTILE OF *AVENA SATIVA*. MATERIAL PLANTED ON  
DECEMBER 4. TEMPERATURE 16° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 $\mu$ )
	<i>amp.</i>			
1 .....	0		2: 00 P.M.	
2 .....	0		2: 20 "	2.0
3 .....	0		2: 40 "	3.0
4 .....	0		3: 00 "	3.0
5 .....	0*		3: 20 "	3.5
6 .....	$0.3 \cdot 10^{-6}$	↓	3: 40 "	3.5
7 .....	"	↓	4: 00 "	2.0
8 .....	"	↓	4: 20 "	2.0
9 .....	"	↓	4: 40 "	1.5
10 .....	"	↓	5: 00 "	1.5

Average 3.2

Average 1.8

\* Current on.

TABLE XIV

EXPERIMENT, DECEMBER 13, 1934. COLEOPTILE OF *AVENA SATIVA*. MATERIAL PLANTED ON DECEMBER 7. TEMPERATURE 16.5° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 $\mu$ )
	<i>amp.</i>			
1 .....	0		5: 00 P.M.	
2 .....	0		5: 20 "	1.5
3 .....	0		5: 40 "	1.5
4 .....	0		6: 00 "	2.0
5 .....	0*		6: 20 "	2.5
6 .....	$0.3 \cdot 10^{-6}$	↓	6: 40 "	2.0
7 .....	"	↓	7: 00 "	2.0
8 .....	"	↓	7: 20 "	2.0
9 .....	"	↓	7: 40 "	2.0

\* Current on.

TABLE XV

EXPERIMENT, DECEMBER 16, 1934. COLEOPTILE OF *AVENA SATIVA*. MATERIAL PLANTED ON DECEMBER 10. LENGTH OF COLEOPTILE 20 MM. TEMPERATURE 16° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 $\mu$ )
	<i>amp.</i>			
1 .....	0		2: 00 P.M.	
2 .....	0		2: 20 "	2.0
3 .....	0		2: 40 "	3.0
4 .....	0		3: 00 "	4.0
5 .....	0		3: 20 "	4.0
6 .....	0*		3: 40 "	3.5
7 .....	$0.3 \cdot 10^{-6}$	↓	4: 00 "	3.0
8 .....	"	↓	4: 20 "	2.0
9 .....	"	↓	4: 40 "	2.0
10 .....	"	↓	5: 00 "	2.0

\* Current on.

TABLE XVI

EXPERIMENT, DECEMBER 11, 1934. COLEOPTILE OF *AVENA SATIVA*. MATERIAL PLANTED ON DECEMBER 5. TEMPERATURE 16.2° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 $\mu$ )
	<i>amp.</i>			
1 .....	0		5: 00 P.M.	
2 .....	0		5: 20 "	2.5
3 .....	0		5: 40 "	3.0
4 .....	0		6: 00 "	4.5
5 .....	0		6: 20 "	3.5
6 .....	0		6: 40 "	2.5
7 .....	0*		7: 00 "	3.25
8 .....	$0.31 \cdot 10^{-8}$	↓	7: 20 "	2.5
9 .....	"	↓	7: 40 "	2.0
10 .....	"	↓	8: 00 "	1.5
11 .....	"	↓	8: 20 "	1.0

} Average 3.1

} Average 1.5

\* Current on.

TABLE XVII

EXPERIMENT, MAY 14, 1934. COLEOPTILE OF *AVENA SATIVA*. MATERIAL PLANTED ON MAY 11. TEMPERATURE 20° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 $\mu$ )
	<i>amp.</i>			
1 .....	0		1: 00 P.M.	
2 .....	0		1: 20 "	4.0
3 .....	0		1: 40 "	3.25
4 .....	0*		2: 00 "	3.0
5 .....	$0.75 \cdot 10^{-8}$	↓	2: 20 "	2.0
6 .....	"	↓	2: 40 "	1.5
7 .....	"	↓	3: 00 "	0.75
8 .....	"	↓	3: 20 "	1.0†
9 .....	0		3: 40 "	1.25
10 .....	0		4: 00 "	2.75
11 .....	0		4: 20 "	1.25
12 .....	0		4: 40 "	0.75
13 .....	0		5: 00 "	0.5

\* Current on.

† Current switched off.

TABLE XVIII

EXPERIMENT, MAY 20, 1934. COLEOPTILE OF *AVENA SATIVA*. MATERIAL PLANTED ON MAY 16.  
TEMPERATURE 21.5° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 $\mu$ )
	<i>amp.</i>			
1 .....	0		2: 00 P.M.	
2 .....	0		2: 20 "	3.5
3 .....	0		2: 40 "	4.5
4 .....	0		3: 00 "	6.0
5 .....	0*		3: 20 "	5.0
7 .....	"	↓	4: 00 "	4.5
6 .....	$0.56 \cdot 10^{-6}$	↓	3: 40 "	4.5
8 .....	"	↓	4: 20 "	4.5†
9 .....	0		4: 40 "	3.25
10 .....	0		5: 00 "	4.0
11 .....	0		5: 20 "	3.0

\* Current on.

† Current switched off.

These eight experiments (tables XI–XVIII) are sufficient to show that a current of the same strength as in the first 10 experiments but passing in the opposite direction—from the top to the base—does not cause any increase in growth, but gives in most cases the opposite effect, namely, a retardation of growth of the coleoptile. This retardation, as is shown by two of the experiments (tables XVII, XVIII), lasts even after the current is switched off.

The coleoptiles of oats, even in their isolated state, are distinguished by an uneven and changeable growth rate, which makes the effect of external factors upon the growth less distinct. Consequently the writers considered it desirable to verify the results of the oats experiments by using another cereal which has a more uniform growth. As an example there are given here two experiments with rye (*Secale cereale* var. Petkus).

These experiments (tables XIX, XX) show that in this case the effect of the electric current upon growth of the coleoptile is the same as in the experiment with the oats. The current which passes from the base to the apex of the coleoptile causes a definite acceleration of growth of short duration. The current which passes in the opposite direction retards growth to a marked degree, and this retardation lasts for a long time even after the current is switched off.

#### UNINJURED SEEDLINGS

In addition to the experiments with the isolated coleoptiles described above, another series of experiments was carried on with uninjured seedlings



TABLE XIX

EXPERIMENT, MAY 17, 1934. COLEOPTILE OF *SECALIS CEREALIS*. AFTER REMOVAL OF THE PRIMORDIAL LEAF THE COLEOPTILE WAS KEPT IN A DARK ROOM FOR 1 HOUR AND 40 MINUTES. TEMPERATURE 21° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 $\mu$ )
	<i>amp.</i>			
1 .....	0		2: 40 P.M.	
2 .....	0		3: 00 "	5.0
3 .....	0		3: 20 "	4.5
4 .....	0		3: 40 "	5.0
5 .....	0		4: 00 "	4.5
6 .....	0*		4: 20 "	5.0
7 .....	$0.6 \cdot 10^{-8}$	↑	4: 40 "	6.0
8 .....	"	↑↑	5: 00 "	6.0
9 .....	"	↑↑	5: 20 "	5.5
10 .....	"	↑↑	5: 40 "	4.5
11 .....	"	↑↑	6: 00 "	4.5
12 .....	"	↑↑	6: 20 "	4.5
13 .....	"	↑↑	6: 40 "	4.0
14 .....	"	↑	7: 00 "	4.0

Average 4.8

Average 5.0

\* Current on.

TABLE XX

EXPERIMENT, MAY 27, 1934. COLEOPTILE OF *SECALIS CEREALIS*. MATERIAL PLANTED ON MAY 23. LENGTH OF COLEOPTILE 34 MM. TEMPERATURE 20.5° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 $\mu$ )
	<i>amp.</i>			
1 .....	0		5: 40 P.M.	
2 .....	0		6: 00 "	5.5
3 .....	0		6: 20 "	5.5
4 .....	0		6: 40 "	7.0
5 .....	0*		7: 00 "	7.0
6 .....	$0.56 \cdot 10^{-8}$	↓	7: 20 "	4.5
7 .....	"	↓↓	7: 40 "	4.5
8 .....	"	↓↓	8: 00 "	4.0
9 .....	"†	↓↓	8: 20 "	4.0
10 .....	0		8: 40 "	2.25
11 .....	0		9: 00 "	3.25
12 .....	0		9: 20 "	3.25

Average 6.5

Average 4.2

\* Current on.

† Current switched off.

of oats, the roots of which were immersed in the water, while the lower part of the coleoptile was fastened in the cut of the cork plate, which covered the water container. The silk thread of the lower electrode was connected with the basal part of the coleoptile above the cork cover in some of the experiments (tables XXI-XXIV), while in other experiments (tables XXV-XXVII) it was immersed directly in the water so that the current passed not only through the coleoptile but also through the mesocotyl and the root system. In all other respects these experiments did not differ from the ones described above.

TABLE XXI

UNINJURED SEEDLING OF *AVENA SATIVA*. TEMPERATURE 24° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 $\mu$ )
	<i>amp.</i>			
1 .....	0		7: 00 A.M.	
2 .....	0		7: 20 "	3.0
3 .....	0		7: 40 "	6.0
4 .....	0		8: 00 "	4.5
5 .....	0		8: 20 "	4.5
6 .....	0*		8: 40 "	4.0
7 .....	$0.98 \cdot 10^{-6}$	↑	9: 00 "	5.5
8 .....	"	↑	9: 20 "	4.5
9 .....	"	↑	9: 40 "	3.5
10 .....	"	↑	10: 00 "	3.0

\* Current on.

TABLE XXII

UNINJURED SEEDLING OF *AVENA SATIVA*. TEMPERATURE 21° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 $\mu$ )
	<i>amp.</i>			
1 .....	0		12: 40 P.M.	
2 .....	0		1: 00 "	4.0
3 .....	0		1: 20 "	6.0
4 .....	0		1: 40 "	5.5
5 .....	0		2: 00 "	6.5
6 .....	0*		2: 20 "	6.0
7 .....	$0.7 \cdot 10^{-6}$	↑	2: 40 "	7.0
8 .....	"	↑	3: 00 "	6.0
9 .....	"	↑	3: 20 "	4.5

\* Current on.

**TABLE XXIII**  
UNINJURED SEEDLING OF *AVENA SATIVA*. TEMPERATURE 24° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 $\mu$ )
	<i>amp.</i>			
1 .....	0		7: 00 A.M.	
2 .....	0		7: 20 "	2.0
3 .....	0		7: 40 "	4.5
4 .....	0		8: 00 "	3.75
5 .....	0		8: 20 "	3.0
6 .....	0*		8: 40 "	4.0
7 .....	$0.98 \cdot 10^{-6}$	↓	9: 00 "	<b>4.0</b>
8 .....	"	↓	9: 20 "	3.0
9 .....	"	↓	9: 40 "	2.5

\* Current on.

**TABLE XXIV**  
UNINJURED SEEDLING OF *AVENA SATIVA*. TEMPERATURE 24° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 $\mu$ )
	<i>amp.</i>			
1 .....	0		9: 00 A.M.	
2 .....	0		9: 20 "	4.0
3 .....	0		9: 40 "	4.5
4 .....	0*		10: 00 "	5.0
5 .....	$0.98 \cdot 10^{-6}$	↓	10: 20 "	<b>4.5</b>
6 .....	"	↓	10: 40 "	4.5
7 .....	"	↓	11: 00 "	4.0

\* Current on.

**TABLE XXV**  
UNINJURED SEEDLING OF *AVENA SATIVA*. TEMPERATURE 21° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 $\mu$ )
	<i>amp.</i>			
1 .....	0		3: 00 P.M.	
2 .....	0		3: 20 "	4.0
3 .....	0		3: 40 "	3.0
4 .....	0		4: 00 "	3.0
5 .....	0*		4: 20 "	3.0
6 .....	$0.56 \cdot 10^{-6}$	↑	4: 40 "	3.0
7 .....	"	↑	5: 00 "	<b>5.0</b>
8 .....	"	↑	5: 20 "	3.0
9 .....	"	↑	5: 40 "	3.0
10 .....	"	↑	6: 00 "	2.5

\* Current on.

**TABLE XXVI**  
UNINJURED SEEDLING OF *AVENA SATIVA*. TEMPERATURE 20° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 $\mu$ )
	<i>amp.</i>			
1 .....	0		4: 20 P.M.	
2 .....	0		4: 40 "	4.0
3 .....	0		5: 00 "	3.0
4 .....	0		5: 20 "	4.0
5 .....	0		5: 40 "	3.0
6 .....	0*		6: 00 "	4.0
7 .....	$0.7 \cdot 10^{-6}$	↑	6: 20 "	5.0
8 .....	"	↑	6: 40 "	3.5
9 .....	"	↑	7: 00 "	3.5

\* Current on.

**TABLE XXVII**  
UNINJURED SEEDLING OF *AVENA SATIVA*. TEMPERATURE 20° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 $\mu$ )
	<i>amp.</i>			
1 .....	0		11: 40 A.M.	
2 .....	0		12: 00 M.	3.0
3 .....	0		12: 20 P.M.	3.0
4 .....	0		12: 40 "	5.0
5 .....	0		1: 00 "	5.0
6 .....	0*		1: 20 "	4.5
7 .....	$0.7 \cdot 10^{-6}$	↓	1: 40 "	4.0
8 .....	"	↓	2: 00 "	4.0
9 .....	"	↓	2: 20 "	3.0

\* Current on.

A comparison of the data of the results of these experiments with the results of the previous ones shows that the same general regularity exists in both cases: a current of the order of  $10^{-7}$  to  $10^{-6}$  amperes caused an acceleration of growth of short duration when it passed from the base to the apex of the plant. When a current of the same strength passed in the opposite direction it either did not effect growth at all, or it caused a slight retardation of growth.

### Conclusions

What are the general conclusions that may be drawn from these experiments? Do they confirm the assumption that the translocation of the growth

hormone in the coleoptile depends on electric forces and that this substance, an acid, moves through the cells of this organ primarily by means of "cataphoresis" as WENT (12) and KÖGL (8) consider it to spread? A superficial observation of the results of our experiments makes one think that they fit easily into this hypothesis. We see that a current passing from the base of the coleoptile to its apex really caused a definite acceleration of growth, and on the contrary the current passing in the opposite direction caused a decrease. But a further analysis of the same data shows that the phenomena observed by us are of a more complicated nature.

As to the acceleration of growth under the influence of the current which passed in the upward direction, it is to be noticed first of all that this acceleration lasts only for a very short time and takes place shortly after the current is switched on. Soon thereafter the growth rate decreases to the normal level and sometimes there is even a retardation in growth. Only in a few cases, where the initial growth of the coleoptile for some reason (*e.g.*, low temperature) was very slow, as in some of the experiments (tables VI-VIII), the electric current seems to have stimulated growth of this organ, increasing the rate of growth to the normal level.

The data relating to the effect of current which passed from the top of the coleoptile to its base seem to agree more closely with the WENT-KÖGL's ideas. The more lasting retardation of growth, which was observed in this case, may be explained by the immediate influence of the current upon movement of the growth hormone. But with this consideration in mind it is hard to understand the phenomenon of "aftereffect" of the current, namely, the decrease in rate of growth after the current is switched off, as seen in the results of the experiments given in tables XVII and XVIII and especially in table XIX. On the basis of the WENT-KÖGL hypothesis one would expect that after the elimination of the cause, which was retarding the normal movement of the growth hormone, its translocation in the growing zone of the coleoptile should at once increase, and this should cause a marked acceleration in growth.

Summing up our results we must say that although our experimental data do not prove the incorrectness of the view that the growth hormone has a tendency to diffuse in the plant from the negative pole to the positive one, it is difficult to coordinate them with the ideas of WENT and KÖGL. By analyzing the general features of the phenomena, which we have observed, we have a greater reason to suppose that the electric current influences the movement of the growth hormone not directly as an electrolyte, but through the complex system of the living protoplasm, upon the changeable properties of which depend both the translocation and the production of the growth substance. Our data seem to point to the complexity of the problem and suggest the need for further investigations.

### Summary

1. A critical review of the recent literature on the electrophysiology of growth emphasizes a number of defects in the methods used by previous authors. The fact is stressed that polarizable electrodes must not be employed when one is working with living plants.

2. The main object of the present work was to check WENT's and KÖGL's hypothesis, which assumes that the translocation of the growth-substance in plant organs is a phenomenon of cataphoresis (8, 12).

3. It was found that the passage through the isolated coleoptile of oats and rye of a current of the order  $10^{-7}$  to  $10^{-6}$  amperes, directed from the base of the coleoptile to its apex, caused a noticeable acceleration of growth, which lasted for a short time and in most cases was followed by a definite retardation of growth.

4. If the current is increased by an initial strength during the experiment, the curve of growth rises again for a short time (tables IX, X).

5. At the lower temperatures of our experiments an electric current, which is directed to the apex of the coleoptile, sometimes does not decrease growth, but increases it somewhat, bringing growth to its normal level (tables VI–VIII).

6. A current of  $10^{-7}$  to  $10^{-6}$  amperes, directed from the apex of the coleoptile to its base, in most cases retards growth of the coleoptile (tables XI–XVIII, XX). This retardation continues after the current is switched off (tables XVII, XVIII).

7. Phenomena entirely analogous to the ones mentioned in paragraphs 3 and 6 of this summary were also observed in uninjured seedlings of the oats when an electric current of the order  $10^{-7}$  to  $10^{-6}$  amperes passed through the coleoptile or through the whole plant (tables XXII–XXVII).

8. The experiments described above do not confirm the hypothesis of WENT and KÖGL. It is difficult to harmonize their ideas with the fact that the acceleration of growth is observed only immediately after the current is on and that it lasts for a very short time. Likewise it is difficult to explain the phenomenon of aftereffect, i.e., the retardation of growth after the current is switched off, by the hypothesis of these authors.

9. The results of this work seem to confirm the conclusion that the electric current affects the translocation of the hormone, not directly as an electrolyte, but indirectly through the complex system of the living protoplasm.

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# ELECTRICAL POLARITY AND AUXIN TRANSPORT

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(WITH TEN FIGURES)

## Introduction

The polar basal transport of the growth substances (auxins, growth hormones) in plants is a well known phenomenon, demonstrated first by WENT (81), and studied in detail by VAN DER WEIJ (78, 80). These investigators used the *Avena* (oat) coleoptile.<sup>1</sup> That the phenomenon is more or less general is indicated by the polar transport of auxin in roots (CHOLODNY, 19; NAGAO 60); in hypocotyls of *Raphanus* (VAN OVERBEEK, 62), of *Pisum* (SKOOG, 74); in leaves (AVERY, 2); in the coleoptile of *Avena* (WENT, 81; LAIBACH and KORNMAN, 40; VAN DER WEIJ, 78, 80; and SKOOG, 74) and corn (VAN OVERBEEK, 63); in *Elaeagnus* (woody cutting) (VAN DER WEIJ, 79); in stems of *Coleus*, *Vicia*, and *Phaseolus*; and in hypocotyls of *Vicia*, *Phaseolus*, and *Lupinus* (MAI, 54).

Other workers have reported non-polar transport of auxin in plants. HITCHCOCK and ZIMMERMAN (31) and ZIMMERMAN and WILCOXON (85) have shown an apical transport of heteroauxin (indole-3-acetic acid) and several other active compounds in stems of *Helianthus tuberosus*, *Nicotiana tabacum*, and in *Lycopersicum esculentum*, as indicated by induction of adventitious roots and by epinastic response of leaves. LOEHWING and BAUGUESS (45) have shown that heteroauxin could be absorbed by the root system of potted seedlings of *Matthiola incana* and be transported apically to increase the stem elongation over that of the controls. Both the Boyce-Thompson workers and LOEHWING and BAUGUESS have merely shown that auxin applied in high concentrations can be carried in the transpiration stream. This, of course, will not give polar transport. Higher concentrations of auxin may have effects which are not normally encountered. For example, high concentrations applied at the base of *Pisum* cuttings induce roots there, whereas in the lower, more physiological concentrations roots may be induced at the bases, only by applying auxin at the morphological tips (WENT and THIMANN, 83).

LAIBACH and FISCHNICH (41) have shown that the transport of heteroauxin was not strictly polar in leaves of *Coleus* and in cotyledons of *Cucumis sativa*, but that transport could occur apically. The apical transport was much smaller than basal transport, however. AVERY (2), as mentioned above, found only basal transport. AVERY determined this by diffusion of

<sup>1</sup> Coleoptiles are leaf-sheaths which envelop growing points and first foliage leaves of grass seedlings.



the auxin occurring naturally in the leaves, whereas LAIBACH and FISCHNICH applied heteroauxin in a concentration of 0.5 per cent. in lanolin, an altogether unphysiological concentration.

JOST and REISZ (34) demonstrated apical transport of high concentrations of heteroauxin in *Avena* coleoptiles. This was seen from the growth of sections with their basal ends immersed in the auxin solutions, and from actual transport experiments in which auxin was collected in agar blocks at the apical ends of sections supplied with auxin in agar at the basal ends. The apical transport was, however, much less pronounced than the normal basal transport, and hence semipolarity still exists. The concentrations of auxin used in the transport experiments was 1:200,000, the length of the sections 10 mm., and the time of transport overnight. As to its effect on growth, the apical transport may have been effected by capillarity in the hollow coleoptiles. Regardless of these findings, WENT, VAN DER WEIJ, and others always observed strictly basal transport in short sections (1 to 4 or 5 mm.) when shorter periods of time (1 to 3 hrs.) were used. Moreover, as VAN DER WEIJ (78) mentioned, when higher concentrations of auxin are used (1:200,000), the auxin may be transported by capillarity in films of water condensed on the surfaces of the sections. At any rate, although strict polarity is always difficult to observe when high concentrations of auxin are used, the polarity still dominates apical transport. The concentrations ordinarily used are of the order of  $1:10^6$  or less (WENT and THIMANN, 83).

As has been mentioned, CHOLODNY (19) and NAGAO (60) demonstrated a polar basal transport in roots. GORTER (28), DE HAAN (30), and others claim otherwise. These controversial statements will not be discussed at this point, since they have no bearing on the polar basal transport in *Avena*.

### The mechanism of polar transport

The mechanism of this polar transport is as yet little understood. WENT (81) showed that the transport was always in a basal direction in the *Avena* coleoptile when physiological concentrations were used; that in his special case, the velocity of this transport was about two hundred times greater than that of ordinary diffusion (being 10 mm. per hour); and that its polarity was unaffected by gravity. VAN DER WEIJ (78, 80) confirmed these findings and, in addition, showed that the transport would occur against a considerable concentration gradient, suffering no appreciable change. He also found that the velocity of the transport was reversibly lowered to that of diffusion when the temperature was lowered to 0° C. At this temperature, however, the polarity of the transport persisted. Polarity, on the other hand, was reversibly abolished by ether narcosis (VAN DER WEIJ, 80). BONNER (6, 7) indicated that transport was dependent

upon the presence of oxygen. With regard to the independence of transport and gravity, PFAELTZER (64) found that  $14.5 \times$  gravity, produced by a centrifugal field, had no effect on polar transport in the *Avena* coleoptile.

#### PROTOPLASMIC STREAMING AS THE MECHANISM

VAN DER WEIJ (70) concluded that polar transport was a "vital process," but that protoplasmic streaming had nothing to do with it, since the velocity of transport was independent of temperature down to very low values, e.g.  $0^{\circ}\text{C}$ ., while the velocity of protoplasmic streaming depended upon temperature within wide limits, citing LAMBERS (42). This view is supported by the observation of SCHUMACHER (73) that fluorescein shows polar diffusion in the plasma of stem hairs of *Cucurbita pepo*, the rate and direction of this transport being constant, while the rate and direction of protoplasmic streaming varied. BOTTELIER (9) favored some correlation between protoplasmic streaming and transport, finding the *velocity* of streaming (3 cm. per hour) to be *constant* between  $17^{\circ}$  and  $35^{\circ}\text{C}$ ., while the amount of protoplasm in actual rotation (streaming intensity) increased with temperature, just as transport intensity increases (VAN DER WEIJ, 78). Furthermore the velocity of transport (1 cm. per hour) is too great to be explained by a diffusion process unless it is of the nature of the model described by VAN DEN HONERT (cf. below). BOTTELIER (10) also found that oxygen limited protoplasmic streaming, as it presumably does transport.

From this discussion it is probable that protoplasmic streaming has nothing to do with the *polarity* of transport, but may be a factor in its *velocity*.

#### ACTIVATED DIFFUSION AS THE MECHANISM

A possible mechanism for transport is that suggested by several workers (cf. BRINKMAN and SZENT-GYÖRGYI, 16; VAN DEN HONERT, 32; SÖLLNER, 75), which demonstrates the transport of surface-active substances at interfaces whose interfacial tension has been lowered at one end by the addition of these substances ("spreading"). The velocity of the transport of KOH in VAN DEN HONERT's model was in one case 68,000 times greater than that of ordinary diffusion of KOH.

MASON and MASKELL (57) found that the diffusion of sugar in the cortex of the cotton plant complies with the rules of concentration gradients and directional flow for diffusion, but that the velocity was between 20,000 and 40,000 times greater than the ordinary diffusion of sugar would be expected to exhibit. MASON and PHILLIS (58) found that oxygen was necessary for such transport in the cotton plant, and state: "It is suggested that the mechanism activating diffusion consists in some special organization in the cytoplasm, maintained by metabolic energy, whereby the resistance to solute movement is so reduced that materials diffuse in the sieve-tube at rates com-

parable with those in a gas." PHILLIS and MASON (66), moreover, have shown that sucrose is transported against a concentration gradient in the leaf of the cotton plant. This recalls the similar transport of auxin against a concentration gradient in the *Avena* coleoptile, but is different in that the sugar transport is not as polar. The "organization" spoken of by MASON and PHILLIS is as yet unknown, although the model of VAN DEN HONERT is suggestive.

Hence "activated diffusion" may be a factor in determining the velocity of auxin transport in plants, particularly since auxin is surface active (OKUNUKI, 61; KÖGL, ERXLEBEN, and HAAGEN-SMIT, 39), but it is difficult to see how such a mechanism could explain polarity.

### Electrical polarity

BRAUNER (13) found that the underside of horizontally placed plants became electropositive to the upper side. He also found that the shaded side of illuminated seedlings developed an electropositivity with respect to the illuminated side. Later BRAUNER and BÜNNING (15) correlated the geo-electric effect with electrotropisms. CHOLODNY (18) had already developed the theory that the plant growth hormone is electrically transported in the plant, accumulating more on one side than on the other, thus causing differential growth and a tropism. DOLK (22) assumed that the growth hormone was an acid, and suggested that the dissociated anion would be transported to the geo- or photo-induced positive pole. For a review of this literature, see WENT (82).

From a survey of older literature on the subject of electrical polarity in living organisms, WENT (82) formulated in his "Botanische Polaritätstheorie" the idea that the dissociated anion of auxin is transported longitudinally in the plant as a result of the inherent electrical polarity of the organ in question. By this time it was known that auxin was a weak acid (KÖGL and HAAGEN-SMIT, 38). Applying his theory to seedlings, WENT suggested that the apical end of a seedling was electronegative to the basal end, and that auxin anions were electrically and polarly transported basalward. He supported this theory with experiments demonstrating that *Impatiens* cuttings exhibited a bipolar staining in acid and basic dyes (cf. experimental part of this paper).

Previous indirect evidence has seemed to favor a causal relation between lateral transport of auxin and bioelectric potentials in plants (BRAUNER and BÜNNING, 15; AMLONG, 1; KOCH, 37; DE HAAN, 30).

For a general discussion of transport and of the polarity theories, the reader is referred to BOYSEN-JENSEN (12) and WENT and THIMANN (83).

In conclusion, it may be said that evidence seems to favor both protoplasmic streaming and activated diffusion as velocity components in trans-

port, and bioelectric potentials as the cause of polarity in the transport of auxin in plants.

It is the purpose of the present papers to reexamine the possibility of a linkage between electrical polarity and the polar transport of auxin in plants, the first paper being concerned with electrical polarity, and the second (see July, 1937, issue) with transport and electrical polarity.

#### A. INHERENT ELECTRICAL POLARITY

1. POLAR DYE UPTAKE.—As mentioned in the introduction, WENT (81), to substantiate his electrical polarity theory, demonstrated a bi-polar uptake of dyes in *Impatiens* cuttings. Negatively charged (acid) dyes penetrated most at the cut apices, and positively charged (basic) dyes at the bases of immersed cuttings. Referring to his paper, it is seen that his acid dyes included light-green, acid green, quinolin yellow, and methyl orange; while the basic dyes included safranin, methyl violet, prune pure, neutral red, thionin, and gentian violet. DE HAAN (30) investigated differential staining in geotropically bending *Vicia* roots and found that basic dyes accumulated most on the convex side, as would be expected from BRAUNER'S (13) finding that this side was electropositive to the concave side. DE HAAN classified light-green and methyl orange as "anode-coloring" (basic) which contradicts WENT'S classification.

With respect to such controversies, electrophoretic experiments were performed with all of the dyes used in the experiments about to be described. The dye solutions (0.5 per cent.) were made up in distilled water at pH 6.0, and a current passed from zinc electrodes through U-tubes containing the dyes. The products of electrolysis at the electrodes were washed away by automatic siphons during the current passage. At the end of the experiments, the pH values at the electrodes were found to be unchanged. When such experiments were performed, all dyes classified as acids were found to be cathodic and all basic dyes anodic. KELLER has said, however, that dye particles reverse their charges in protoplasm due to the fact that colloids adsorb the particles and impart to them the charge of the colloidal particle (KELLER, 35, 36; GICKLHORN and KELLER, 27). An exception exists, he says, when the dye is in such excess that the charge of the adsorbed dye particles neutralizes that of the colloidal particle. In this case the charge of the dye particle is not reversed. LAUER (43) could not confirm KELLER'S claim that protoplasm reverses the dye-particle charge. DE HAAN himself found a lack of agreement between KELLER'S tables and "test-object" *Hedera helix*, used by KELLER in making up these tables. Owing to such uncertainties, it was assumed that the present electrophoretic experiments, conducted at pH 6.0 (which is approximately that of the cell content in plants), gave the true charge of the dyes used in the following experiments with plant cut-

tings. It was likewise assumed that the plant did not alter the sign of the charge on the particles. Electrical measurements later bore out this last assumption.

PRINGSHEIM (67) criticized WENT's dye-uptake experiments in that the distances penetrated (1 mm. at best) observed by WENT could not allow conclusions to be drawn. According to this criticism, DE HAAN's observations, based on the number of cells stained in cross sections of *Vicia* roots, would be even less valid. From such considerations, it became of interest to repeat WENT's experiments and to test these findings with electrical measurements. The following descriptions show that WENT's observations can be clearly duplicated.

TABLE I

BIPOLAR DYE UPTAKE IN *IMPATIENS* CUTTINGS

## KEY:

0 .....	No staining
2 .....	Slight staining
4 .....	Up to 0.5 mm. penetration
6 .....	1 mm. penetration
8 .....	1.5 mm. "
10 .....	2.0 mm. "
14 .....	3.0 mm. "

DYE	PENETRATION	
	APEX	BASE
Negative dyes		
Trypan blue .....	2.0	3.0
Light-green .....	7.0	0.5
Methyl blue .....	5.0	2.0
Methyl orange .....	7.0	2.5
Congo red (colloidal) .....	4.5	6.5
Orange-G .....	10.0	1.0
Average .....	6.0	2.6
Positive dyes		
Safranin .....	7.0	9.3
Methyl violet .....	5.0	11.0
Neutral red .....	3.5	11.0
Janus green .....	5.0	9.0
Bismark brown .....	4.0	9.0
Thionin .....	5.0	7.0
Methylene blue .....	3.5	12.5
Cresyl violet .....	4.0	8.5
Brilliant cresyl blue .....	6.0	11.0
Nile blue A .....	8.0	14.0
Average .....	5.1	10.2

Etiolated *Impatiens balsamina* seedlings grown in sand in the dark room at constant temperature and humidity, were prepared by cutting away the cotyledons and the parts underground. Two to three cut hypocotyls were placed in upright test tubes filled with the dye solutions in 1 per cent. sucrose. The experiments were run in the dark 15 to 24 hours. After this time the hypocotyls were removed and examined in daylight, the amounts of penetration being noted and recorded as shown in table I. The concentrations of the dyes used were from 0.1 per cent. down to 0.001 per cent. In the case of the basic dyes, the higher concentrations caused more rapid infiltration of the tissue. In the case of the acid dyes, the lower concentrations frequently showed little or no staining at the apices and bases of the hypocotyls. The results of one experiment are summarized in table I, sections showing indistinguishable staining or infiltration being discarded. Three other experiments, run at other times, showed essentially the same thing, so that the table represents a typical case, and a confirmation of WENT's experiments. The numerals are represented on the same scale as in WENT's tables for purposes of comparison.

For purposes of comparison, WENT's averages for negative dyes were: apex 2.5, base 1.1; for positive dyes, apex 2.4, base 3.7. It is noteworthy that the greatest penetration WENT obtained was, at the most, one millimeter; while as much as three millimeters penetration was observed in the present work. Presumably sucrose in the solutions maintained oxidations and kept the tissues in a more normal condition during the time they were immersed.

RAMSHORN (68) criticized WENT's conclusions from such dye experiments, on the ground that actual electrical measurements of *Impatiens* cuttings showed electropositivity of the cut apices with respect to the cut bases. Upon repeating such measurements, measuring the dye uptake at the same time, quite the opposite was found. The experiment below is typical:

*Impatiens* hypocotyls were cut and placed horizontally with each cut end in a cup of the dye solution in SHIVE's solution made up to 1 per cent. in sucrose. Agar-0.1 N KCl bridges from each cup led to a 0.1 N KCl solution in a cup in which the side-arm of a Zn-saturated ZnSO<sub>4</sub> half-cell could be placed. The potential differences between apex and base were measured with the WULF string electrometer described later in this paper. Table II shows the potential differences (P.D.'s) expressed in millivolts (mv.), the polarity being expressed as the sign of the tip with respect to the base. The dye penetration is represented in millimeters, the recorded figures being the averages. Several (3 to 5) sections were used for each dye solution, hence the electrical measurements represent the average of the several cuttings in parallel circuit. The dye concentrations were 0.05 per cent. All dye-charges were rechecked electrophoretically. The cuttings varied from 6 to 10 mm. in length.

In other experiments, the P.D.'s were read frequently over a period of several hours. It was revealed that the apex is at first positive<sup>2</sup> with respect to the base. In two hours after setting up the experiment it is electro-negative, and remains so until the tissues appear abnormal (flaccid), after which the P.D. drops toward zero. This time-relation in establishing electrical polarities will be discussed in more detail under the section on P.D. gradients.

These electrical measurements confirm the dye-uptake experiments, and thus the polarity first claimed by WENT is real; that is, the apex of the *Impatiens* hypocotyl is electronegative to the base.<sup>3</sup>

RAMSHORN's conflicting results may be explained by the time-relations in establishing the normal electrical polarity. It will be noticed that the apical negativity did not appear at once, but two hours or more elapsed before the tip became negative.

TABLE II

MEASURED ELECTRICAL POLARITY AND BIPOLAR DYE UPTAKE IN *IMPATIENS* HYPOCOTYLS\*

DYES USED	POTENTIALS		DYE UPTAKE	
	6: 00 P.M.	12 HR. LATER	APEX	BASE
	mv.	mv.	mm.	mm.
Negative dyes				
Light-green .....	+ 11.0 (tip +)	- 14.0 (tip -)	1.5	0.0
Methyl blue .....	+ 13.0	- 5.0	trace	trace
Methyl orange .....	+ 12.0	- 7.0	7.0	5.0
Methyl orange-G .....	+ 15.0	- 5.0	1.5	0.0
Average .....	+ 12.7	- 7.7	2.4	1.2
Positive dyes				
Safranin .....	+ 9.0	- 7.0	trace	1.5
Methyl violet .....	+ 7.0	- 12.0	1.0	2.0
Bismark brown .....	+ 3.0	- 3.5	trace	trace
Methylene blue .....	+ 1.0	- 1.5	0.5	1.5
Cresyl violet .....	+ 6.5	- 2.0	1.0	2.0
Brilliant cresyl blue	+ 9.0	- 9.0	trace	trace
Nile blue A .....	+ 6.0	- 9.5	1.2	2.5
Auramine .....	+ 9.0	- 6.0	5.0	7.0
Average .....	+ 6.3	- 6.3	1.1	2.1
Controls				
Shive's solution .....	+ 8	- 12	.....	.....
Crone's solution .....	+ 7	- 20	.....	.....

\* The hypocotyls were cut and placed in the cups at 5: 00 P.M. (3 per dye).

<sup>2</sup> In this paper the electrical polarity is expressed with respect to the external circuit.

<sup>3</sup> The possibility remains that the electrical polarity revealed by dye uptake and the polarity revealed by measurements are alike by coincidence.

## 2. MEASURED ELECTRICAL POLARITY.—

a. *Brief review of the literature.*—Many attempts have been made to correlate morphological and physiological polarity with electrical polarity in living organisms (cf. RAMSHORN, 68; and WENT, 82). CHILD's school (CHILD and HYMAN, 17; HYMAN and BELLAMY, 33) claimed that the regions of highest metabolic rate in hydroids (apical regions) may be electronegative to other regions: LUND (47, 50), and LUND and KENYON (53), on the other hand, claimed that electrical polarity was dependent upon oxidation-reduction potentials (cf. discussion). Usually parts of polar tissues (apical end of hydroid stems, onion root tips) having the highest rates of oxidations were electropositive to other regions. RAMSHORN (68) stated that in seedlings of several different plants, electropositivity was directly linked with growth rate, such that potential distributions paralleled growth rate distributions. BARTH (3) showed that in several different hydroids the electrical polarity varied, some hydroids exhibiting apical electronegativity while others showed positivity. It is difficult to make *generalities* from such conflicting statements.

In general, the cortex of a root apex is held to be normally electropositive to that of the base (LUND and KENYON, 53; MARSH, 55, 56; RAMSHORN, 68). In hypocotyls and coleoptiles of seedlings, the apical cortex is said to be electropositive to the basal (RAMSHORN, 68). In the Douglas fir, the apical cortex is as RAMSHORN claimed to be the case in seedlings, *i.e.*, electropositive with respect to basal; while the apical wood is electronegative to the basal (LUND, 48, 49, 51). In seedlings with internodes, in general, the nodal regions are electropositive to internodal zones; and the total polarity from apex (just below cotyledons) to the base of the stem shows an electronegativity of the tip to base (REHM, 70; CLARK, this paper.) RAMSHORN (68), on the other hand, claimed the opposite, *i.e.*, that the tip was electropositive to the base (cf. discussion).

The following descriptions concern themselves directly with the determination of the electrical polarity of the *Avena* seedling, and of a few other seedlings.<sup>4</sup>

b. *Methods involved.*—In determining the electric potential differences (P.D.'s) in *Avena*, various types of electrodes, contacts, and recording instruments were tried. A Dolezalek electrometer was used as the recording instrument at first, but was found to be difficult of manipulation and to have too long a period. A Compton electrometer is subject to the same criticism, although it is somewhat better than the Dolezalek instrument. Lindemann electrometers are too insensitive. A potentiometer is apt to draw some

<sup>4</sup>Seedlings are preferred to mature, green plants because they have been more extensively studied, are more quickly obtained, and because they can be used in the dark. Light complicates the physiological behavior.



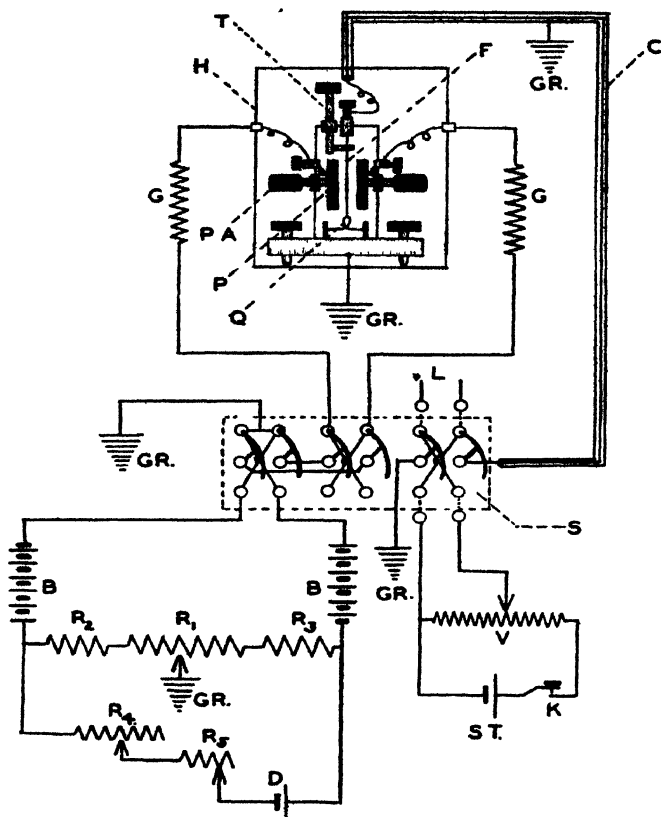


FIG. 1. String electrometer circuit. Key:

- B Six 45-volt heavy-duty Burgess B-batteries (long shelf-life)
- C Copper-tube shielding for fiber lead
- D 1.5-volt dry cell battery
- F Platinum fiber (0.001 mm. diam.)
- G 750,000-ohm wire-wound grid leaks
- GR Ground
- H Constant temperature housing
- K Key
- L Leads to unknown P.D.
- P Plates
- P.A. Plate adjustments
- Q Quartz fiber spring
- $R_1$  10,000-ohm Yaxley wire-wound potentiometer
- $R_2, R_3$  25,000-ohm Yaxley wire-wound potentiometers
- $R_4$  750-ohm Yaxley wire-wound potentiometer
- $R_5$  50-ohm Yaxley wire-wound potentiometer
- S Ceresin-covered mercury-in-paraffin switches
- ST Eppley standard cell
- T Tension adjustment
- V 0.1-10,000-ohm plug-type Welch volt box

current from the living tissues. Hence a WULF string electrometer, constructed in the shops of this institute, was employed for the majority of the measurements (cf. WULF, 84). Figure 1 represents the hook-up.

The sensitivity of the instrument in its final adjustment was more than one millimeter scale-deflection per millivolt with a scale-distance of one meter. Its period was about one second at lower sensitivities. The sensitivity depended upon the diameter of the string (platinum fiber 0.001 mm. in diameter), its tension, the distance between the plates, and the voltage across the plates. At higher sensitivity, damping increased the period to about three seconds. (This could be avoided by housing the instrument in a vacuum). The calibration curves approximated a straight line and remained constant for weeks at a time when the instrument was kept dry and at constant temperature. Because of the constant calibration and rapidity of motion of the string, readings could be made rapidly and accurately.

The distribution of potentials in the *Avena* seedling was first studied by moving contacts up and down the plant. CHAMBERS's micromanipulators (40-pitch threading) were employed to move the electrode contacts.

Electrodes of various types were tried. It was found that bright or platinized platinum electrodes, however cleaned, gave non-reproducible readings, presumably because they were easily unpoised (cf. GICKLHORN, 26; UMRATH, 77; DORFMAN, 24). Ag-AgCl wire loops or claws serving as contacts gave reproducible readings for a while, but demanded frequent replating. Quartz capillaries filled with fresh coagulated egg-white into which Ag-AgCl wire electrodes were set, gave reproducible readings. These were used for obtaining the internal distribution of P.D.'s. The gradients obtained with the Ag-AgCl loops and the quartz micro-electrodes, in general, gave similar results, these results being statistically comparable with those obtained with the more refined glass contacts and unpolarizable electrodes, although there was less constancy. The electrodes finally used for most of the measurements were Zn-saturated  $\text{ZnSO}_4$  half-cells (cf. fig. 2). These remained iso-electric for months at a time. The types of contacts used in conjunction with these electrodes were varied. Usually the electrode was placed in a glass cup filled with the liquid used to make contact with the plant. A glass side-arm connected this electrode cup to the plant. At the point of contact, the side arm was fashioned into a small glass claw or loop, through which the plant led. The glass parts did not usually touch the plant, a meniscus of contact fluid performing this function. Tap water, KCl solutions, distilled water, and various nutrient solutions used in the glass contacts made no difference in the values obtained, if the same fluid was used in all contacts (cf. REHM, 69). AMLONG (1) showed that geo-electric P.D.'s in plants depend upon the concentration of the contact fluids.

The effects of concentration were not determined in the present work, but it was assumed that the P.D.'s measured were not a function of the ionic species of the solution, since any of the solutions gave the same polarity, and approximately the same magnitude of P.D. (cf. REHM). The type of contact used for such measurements is seen in figure 2.

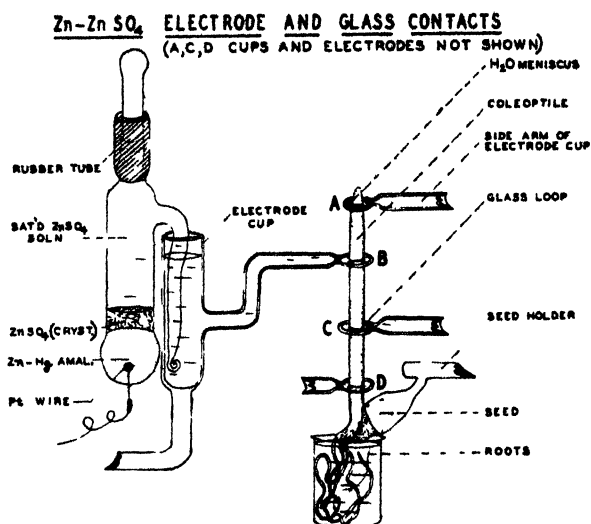


FIG. 2. Diagram of electrode and glass contacts used in measuring the P.D. of *Avena* coleoptiles.

A variation of this type of contact consisted in the use of a cotton or linen thread which was made wet by being threaded through the side arm of the electrode cup to the fluid in the cup. This thread then could either be wrapped around the part of the plant being studied, or could merely touch it (REHM, 69, 70). Such threads maintained an isoelectric condition satisfactorily if they were occasionally washed.

The most useful type of contact, shown in figure 3, consisted in the following: Long, thin agar threads (made up of 2 per cent. agar in tap water, distilled water, 0.1 N KCl, SHIVE's, CRONE's, or HOAGLAND's solution), 0.5 mm. or less in diameter, were made to hang from paraffined glass capillaries filled with the same agar. This was done by pushing an agar-filled capillary into more of the same agar, thus partly displacing the agar in the capillary as a thread. These capillaries were set in larger paraffined glass tubes filled with the same agar, which in turn were mounted in upright rows in a moist chamber. The glass tubes filled with agar led outside the chamber to paraffined electrode cups filled with the solution of which the agar was made.  $Zn-ZnSO_4$  electrodes were placed in these cups. The agar threads hanging from the capillaries made contact to seedlings in the moist chamber,

being held to the plant by a drop of 15 per cent. gelatin. Several such fixed contacts could be made to each plant; and several plants could be set up simultaneously. The threads were prevented from drying by maintaining the chamber at near saturation with water vapor from strips of moist filter paper on the sides of the chamber. This method has the advantage that the agar threads remain fixed to the plant in the same position, being carried by upward growth without stimulating the plant. Seedlings, to which several glass contacts were fixed, frequently grew up through the more apical contacts, necessitating moving the apical contact back up to the tip. Such manipulations, however carefully done, usually stimulated the plant, thereby altering the P.D.'s.

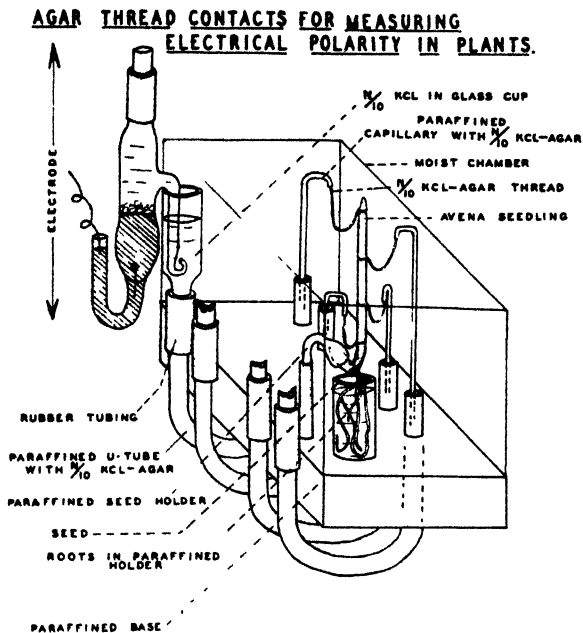


FIG. 3. Diagram of agar-thread contacts for measuring electrical polarity. Note that only one of several plants is shown, and only one of four electrodes is shown in the drawing.

*c. Electrical polarity and P.D. distributions in the Avena coleoptile.*—As described in an earlier paper (CLARK, 20), the P.D.'s in the *Avena* seedling, obtained by manipulation of two contacts up and down the plant, were not constant over any considerable period of time, since the manipulation always resulted in changes of P.D.'s. Such "handling reactions" are illustrated in figure 4. This was usually true regardless of the care taken in making the manipulation, even if contact to the plant was made merely by a meniscus of water from the contacts. Moreover the orange light in the

dark room proved to be a stimulus.<sup>5</sup> Plants left in complete darkness gave variable P.D.'s as soon as this light was again turned on. Again, if two contacts were left on the plant in a fixed position, one at the tip and the other at the base of the coleoptile, and the plant left in complete darkness, con-

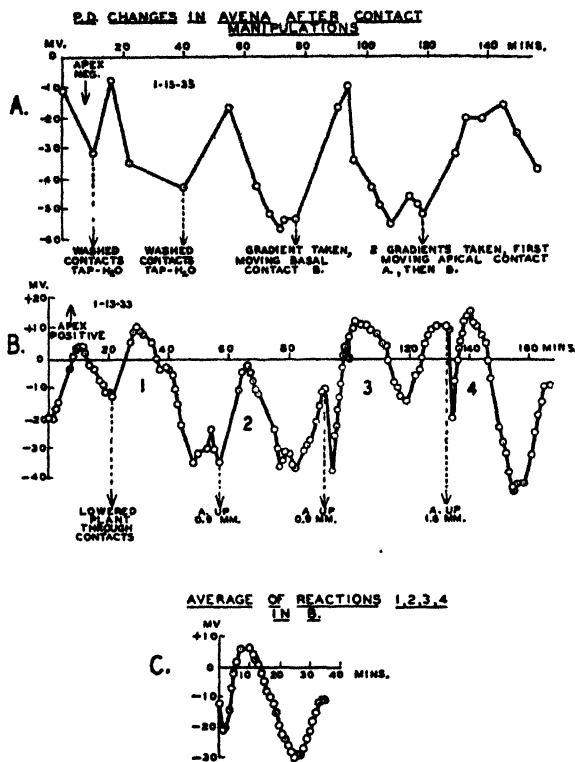


FIG. 4. Changes in P.D. of *Avena* coleoptiles produced by manipulation of contacts.

stancy of the P.D. was established only after an average time of 110 minutes. This constancy was abolished occasionally by the appearance of rhythmical P.D. changes, due probably to growth of the coleoptile up through the contacts, and also due to nutations which cause rubbing against the glass of the contacts. Clean glass adheres rather strongly to the *Avena* coleoptile cuticle, and considerable stimulation is caused by such movements. This rhythmical effect was avoided by the use of glass contacts dipped into 15 per cent. gelatin, thereby rendering the contacts slippery to the cuticle, or by recourse to the agar thread technique described above.<sup>6</sup>

<sup>5</sup> The light used to illuminate the dark room was filtered through a Corning filter no. 348, which cut out all wave-lengths below 575 mμ. No phototropism occurred in this light.

<sup>6</sup> Stimulation by contact is discussed by PFEFFER (65).

To obtain the normal P.D. distribution in the coleoptile, therefore, four fixed contacts were made to the plant (*cf.* fig. 2, A, B, C, D, and fig. 3). The plants were left in complete darkness. After constancy of P.D.'s obtained (80–120 minutes), the P.D. distribution could be easily and quickly determined. Figure 5 A represents the relative constancy of the P.D. between tip and base of *Avena* coleoptiles with fixed, gelatin-dipped glass electrodes; while figure 5 B represents the same type of experiment in which, however, the contacts were not gelatin-dipped.

Figure 5 C shows the much greater constancy obtained when the agar-thread contact method was used. The constancy lasts several hours. The figure has fewer points than either 5 A or B, but that no changes occur between the points has been verified by many other determinations.

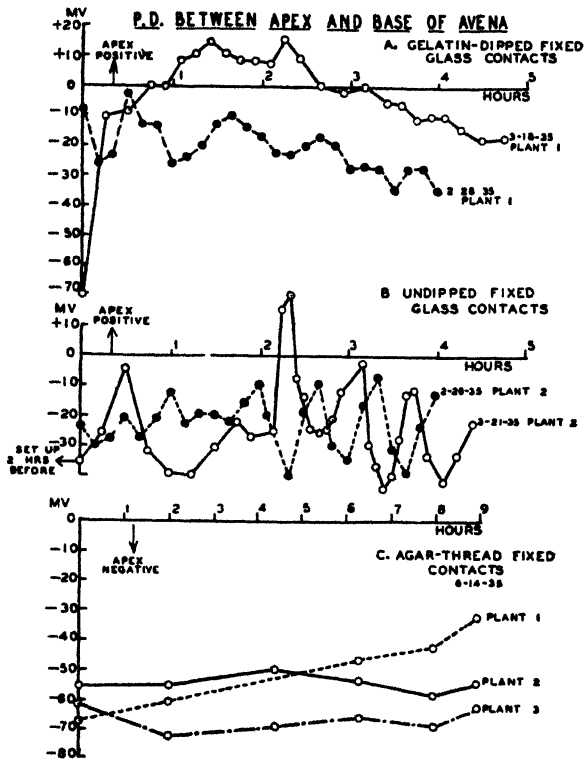


FIG. 5. P.D. between apex and base of *Avena*.

A. P.D. between apex and base of the *Avena* coleoptile, on using glass contacts dipped in gelatin.

B. P.D. between apex and base of *Avena* coleoptile, on using contacts not gelatin dipped.

C. Establishment of constant P.D. between apex and base of *Avena* coleoptile using agar-string contacts.

Figure 6 A illustrates the electrical polarity and P.D. distribution in the *Avena* coleoptile of intact plants as determined with the four fixed, gelatin-dipped glass contacts, the plant being in total darkness, and constancy having been obtained. The electrode cups each contained a Zn-ZnSO<sub>4</sub> electrode, each of which was isoelectric to all the others both before and after each experiment. The P.D. between each contact position to the plant was obtained by manipulation of mercury-in-paraffin switches outside the experimental chamber in which the plants were housed. Figure 6 B illustrates a similar gradient taken with only two glass contacts, the basal contact being fixed, and the apical contact being moved toward the base by means of the micro-manipulators. Every few mm., a reading was taken. After the contacts touched each other, the apical contact was again moved upward, readings being taken every 5 mm. The movements of such fluid contacts up and down the coleoptile did not wet the surface and thus invite electrical shunting (cf. ROSENE, 71), because of the fatty nature of the cuticle. It will be noticed that the gradient taken by the manipulation down the coleoptile differs from that taken on moving the contact back up the coleoptile. After such manipulations it is found that the P.D.'s vary considerably, and sometimes the polarity is reversed for considerable time (cf. figure 4). Figure

#### ELECTRICAL POLARITY AND P.D. DISTRIBUTION IN AVENA

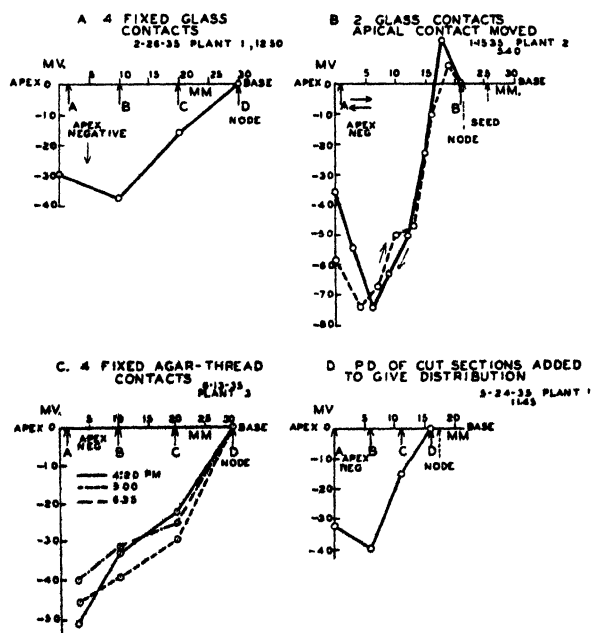


FIG. 6. Electrical polarity and P.D. distribution in the *Avena* coleoptile with various contacts and arrangements.

6 C represents a similar gradient, the contacts in this case being four fixed agar threads as described above. The three curves in C represent three gradients taken on the same plant at different times, showing that the P.D. distribution remained constant for a considerable time. Figure 6 D represents the P.D. distribution calculated from the individual P.D.'s of cut-sections of a coleoptile (cf. section on P.D.'s of cut sections). In all of these curves, electronegativity of the tip is represented on the ordinates, and the length of the coleoptile is represented on the abscissae, A, B, C, and D representing points of contact to the coleoptile from tip to base. The potential at D is taken as the reference zero. The P.D. from A to D is always equal to the sum  $AB + BC + CD$ .

From the above experiments it is clear that the tip of the *Avena* coleoptile is normally electronegative to the base.

d. *Electrical polarity and P.D. distribution in Pisum, Impatiens, and Zea.*—*Pisum sativum* seedlings were studied for the normal electrical polarity and P.D. distribution. Contacts were made to etiolated plants with the linen threads previously described, the threads being wrapped around the zone to be measured. There were several electrode contacts to each plant, represented by the positions on the abscissae corresponding to the points plotted in figure 7 A, 1, 2, 3. The P.D. obtained between each contact was found to be fairly constant for several hours at a time. The measurements were made in the darkroom in weak red light. Figure 7 A represents typical gradients. It will be noticed that nodal zones are electropositive to the internodal zones, and that there exists an electronegativity of the tip with respect to more basal regions. This confirms REHM's (70) findings on *Phaseolus*, and disagrees with RAMSHORN's (68) finding that the tips of *Asparagus* seedlings are electropositive to the basal regions, although the nodal zones were positive to internodal zones.

Etiolated *Impatiens balsamina* seedlings were set up in the same way as *Pisum* seedlings were, and the P.D. distribution recorded. The hypocotyls are without nodes, and the plants were only seven centimeters in height. Figure 7 B represents the distribution found.

*Zea mays* seedlings 5 to 7 cm. in height were set up in the same way as has been described for the *Avena* seedlings, four fixed contacts being made by means of agar threads. The polarity and P.D. distributions found corresponded very closely with those recorded for *Avena*.

It is concluded from these observations that in *Pisum*, *Impatiens*, and *Zea*, the tip of the etiolated seedling is normally electronegative to the basal regions. This shows that the electrical polarity of the *Avena* coleoptile is not unique in its apical negativity.

e. *Internal electrical polarity in the Avena coleoptile.*—The above discussion concerns the electrical polarity and P.D. distribution measured on



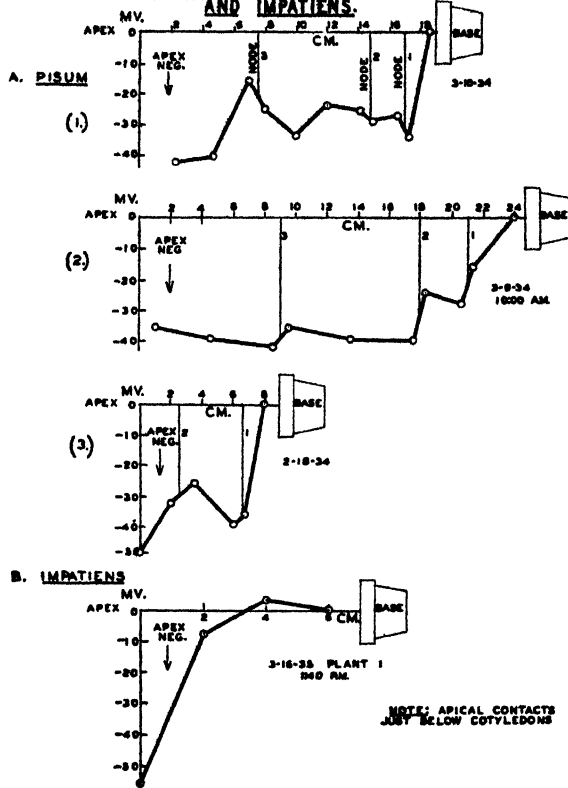
ELECTRICAL POLARITY AND P.D. DISTRIBUTION IN *PISUM* AND *IMPATIENS*.

FIG. 7. Electrical polarity and P.D. distribution in *Pisum sativum* and *Impatiens balsamina*.

the cuticle, thus the *external* polarity. It is conceivable that the distribution of *internal* P.D.'s might be different, as suggested by LUND's (49, 51) findings in the Douglas fir. Here the apical wood was electronegative to basal wood, while the apical cortex was electropositive to basal. For this reason a few experiments were performed on the *Avena* coleoptile, in which the P.D. distribution beneath the cuticle was examined. Quartz microelectrodes (*cf.* descriptions in this paper) were inserted in coleoptiles by means of micromanipulators, and the electrical polarity was measured. The electrodes are illustrated in figure 8. They maintained an isoelectric condition very satisfactorily for two or three hours. Figure 8 A shows the P.D. between two such electrodes inserted in a coleoptile. One electrode was inserted in the wall of the coleoptile at the apex, and one at the base, both electrodes being in the same side-wall, but being inserted from opposite sides of the coleoptile. Traumatopisms toward the sites of insertion occurred

after about 30 minutes, as diagrammed in figure 8 A. Immediately after insertion, the apex of the coleoptile became electropositive to the base, but after 20 or 30 minutes, as seen in figure 8 A, the tip became negative. It was considered impracticable to insert several such electrodes, or to reinsert the same two electrodes at several different loci on the coleoptile in order to obtain the P.D. distribution. This would have resulted in even more P.D. variation than is depicted in the figure; hence, in order to obtain the distribution of internal P.D.'s, a different technique was employed. Several longitudinal slits were made down the coleoptile using a sharp razor. Each slit had its counterpart on the opposite side of the coleoptile in order to compensate the wounding effects. Such coleoptiles will remain straight, whereas the ones in which the microelectrodes were inserted showed traumatropisms. Glass-loop contacts, such as were described above, made contact with the plant, one at the tip and one at the base of the coleoptile. The basal contact was racked up the coleoptile toward the apex by means of the micro-manipulator. When a contact was centered over a slit, presumably the potential internal to the cuticle was measured. Figure 8 C shows the distributions obtained in this way. The vertical lines, a, b, c, d, and e represent the loci of the slits in the coleoptile. Curve 1 represents the distribution obtained by racking the basal contact up to the apex, whereas curve 2 represents the distribution obtained by racking it back down a few minutes later. Curve 3 represents the distribution obtained by racking it back up 20 minutes later. Figure 8 B illustrates the changes in P.D. between the apex and base of the coleoptile before and after each distribution was determined. It will be noticed in curve 2 of figure 8 C, that the readings were taken with the contacts centered on the slits. The distribution is very similar to that obtained on the intact cuticle. Curve 1 shows the same thing, with the exception that one contact was centered *between* two slits, thus on the intact cuticle (between d and e). Curve 3 shows the type of curve obtained when no attention was paid to the position of contacts with respect to slits, i.e., the distribution was taken at more frequent loci, regardless of the slit positions. The general electrical polarity is the same as that on the intact cuticle, but the curves are not smooth. This indicates a *radial* P.D. between cuticle and the internal tissues. The curves are smooth if contacts and slits coincide.

The following section (f) will show that cut sections are of the same electrical polarity as has just been described; and, as seen in figure 6, the sum of the section P.D.'s of a coleoptile give a P.D. distribution for the coleoptile which is comparable to that in an intact plant.

The evidence presented indicates that the distribution of internal P.D.'s in the *Avena* coleoptile is the same as that on the outside (cuticle). The polarity is the same, i.e., the tip is electronegative to the base.

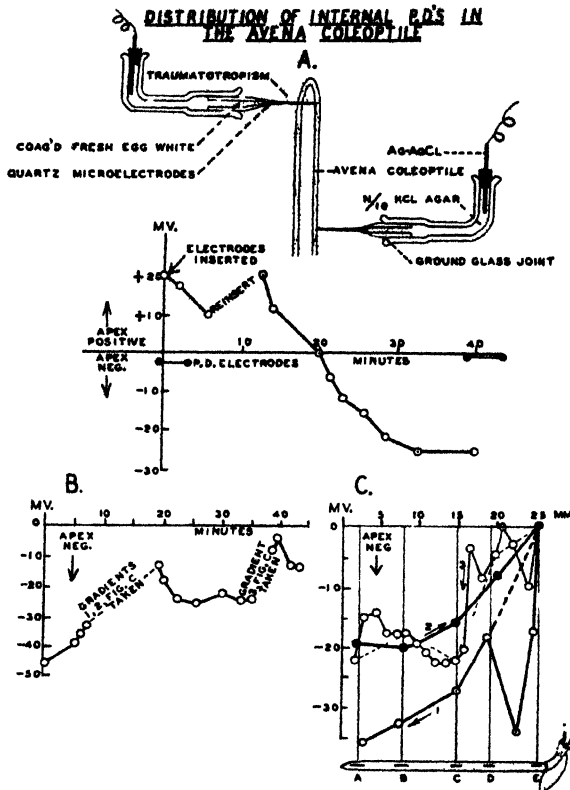


FIG. 8. The internal electrical polarity and distribution of internal P.D.'s in the *Avena* coleoptile.

f. *Electrical polarity of cut sections.*—The P.D.'s and electrical polarity of cut sections of the *Avena* coleoptile were then determined in several different ways. One method involved cutting the sections with two parallel razor blades separated by a brass strip. The cut surfaces were washed by placing the sections upright on wet filter paper for an hour. The sections were then carefully transferred to the experimental chamber, using eye-forceps. Contact was made to individual sections with the agar-thread method, or with agar strips. 0.1 N KCl agar was used in most cases. The strips or threads led through paraffined glass tubes to electrode cups outside the chamber. Zn-ZnSO<sub>4</sub> electrodes were placed in these cups, and the P.D.'s measured. The chamber was maintained at a high vapor pressure by means of strips of moist filter paper.

Several hundred measurements on 3-mm. sections revealed that the cut apical surface was always electronegative to the cut basal surface from 1 to 15 mv., the magnitudes depending upon the time at which the measurements were made.

Another method involved placing from 12 to 20 sections on an agar block, and placing a similar agar block on the tops of the sections. 0.1 N KCl agar strips made contact with these blocks and to the cups outside the chamber. Here, therefore, the average P.D. of several sections in parallel was measured. The same result was obtained, *i.e.*, apical negativity. This latter method will be discussed again in a later paper.

A third method involved making contact with several places on longer sections with agar threads held in place with a drop of gelatin, as described in an earlier section of this paper. The cut surfaces were usually electro-negative to the intact cuticle (another indication of a "radial" polarity), but the apical cut surface was always negative to the basal cut surface.

Using the method by which 20 sections were measured in parallel at the same time, the relation of the length of the section to the P.D. of the section was determined. It was usually found that time was required before the maximum P.D. was established (Presumably this was due to the diffusion of ions from the sections into the agar blocks). For this reason, the P.D.'s were allowed to reach their maximum values before plotting against length. This time function was more pronounced in longer sections. Figure 9

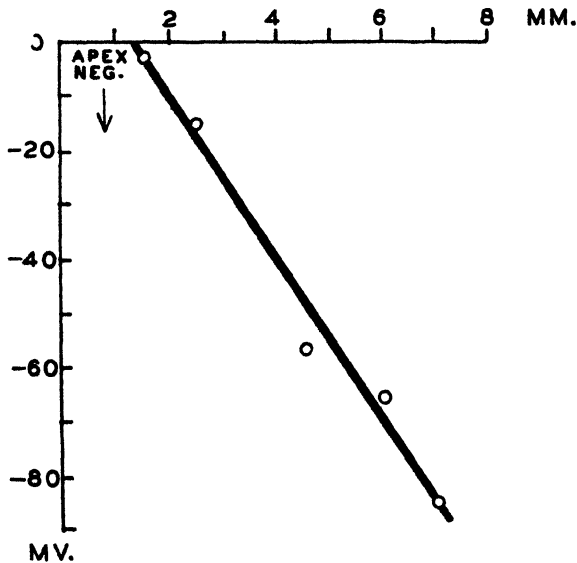


FIG. 9. The relation of section length to P.D.

shows the length of the section in millimeters plotted against P.D. in millivolts, after this maximum P.D. had been reached in all sections (5 hours).

In the section on P.D. distribution in intact plants (fig. 6), it was seen that the sum of the several P.D.'s along a coleoptile was equal to that measured from apical contact to basal contact. In figure 9 it is seen that

this principle of summation again holds, since the magnitude of the P.D. of cut sections is directly proportional to the length of the section. (Cf. LUND, 47, 51, and ROSENE, 71, on the principle of summation of P.D.'s.)

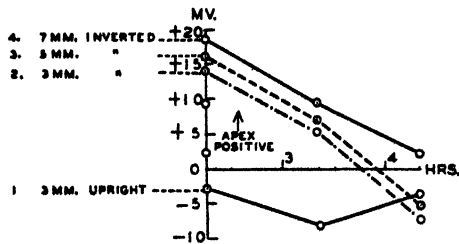
Cut sections of *Vicia faba* and of *Pisum sativum* showed the same electrical polarity as *Avena* coleoptile sections. In the section on dye-uptake, it was seen that *Impatiens* cuttings also showed apical negativity. Thus the phenomenon seems quite general. In *Vicia* and *Pisum*, the P.D. magnitudes varied from a few millivolts to 30 or 40 millivolts, depending upon the length of the section.

*g. Geoelectric effect.*—During the measurements of section P.D.'s, it was noticed that inverted sections exhibited an inverted electrical polarity. A section seemed to show negativity of the end oriented upward regardless of whether this end was morphological tip or base.

The experimental procedure usually consisted in inverting single sections and measuring the individual P.D.'s of these sections, or by placing 12 to 20 sections on one agar block, making contact with this block and with the other cut surface with a similar agar block, thus obtaining the average P.D.'s of the lot of sections in parallel. Measurements were made immediately, when possible, upon inverting the sections. The polarity of the inverted sections showed an immediate inversion of electrical polarity. The time relations of the inversion have not been carefully studied, but the establishment of the inverted polarity seemed to take less time than the geoelectric effects of BRAUNER (13). It was noticed, however, that this geoelectric effect was not maintained indefinitely, particularly in the shorter sections. The original polarity (apical negativity) returned within 60 to 120 minutes, depending upon the length of the sections. Figure 10 A shows the course of the P.D.'s of inverted sections during a period of time. The sections were cut at 9:30 A.M. and placed on wet filter paper in an inverted position. The experiment was set up at 11:30 A.M., the first readings being taken at 11:40 A.M. During this time, the inverted polarity had attained a considerable magnitude.

Figure 10 A, curve 1, shows the change in P.D. of control upright 3-mm. sections. They exhibit normal apical negativity. Curve 2 is for inverted sections 3 mm. in length; curve 3, 5 mm.; curve 4, 7 mm. in length. It is seen that the longer the sections, the greater the magnitude of the inverted polarity; and that the greater the magnitude of this inverted polarity, the longer the time necessary to reestablish the normal polarity. The abscissae are in hours after cutting the sections. Figure 10 B represents data from the same experiment. The P.D.'s of sections with inverted polarity are plotted against the length of the sections in millimeters. The P.D. values used are those of maximum magnitude, *i.e.*, at the first measurements, as represented on the zero ordinate of figure 10 A. It is seen that a nearly

A.  
RETURN OF "NORMAL" POLARITY IN INVERTED SECTIONS



B.  
RELATION OF P.D. TO LENGTH OF INVERTED SECTIONS

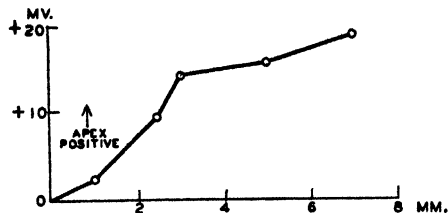


FIG. 10. "Goelectric" polarity of sections.

direct proportionality exists between length and the P.D.'s of inverted polarity, thus indicating a similarity to upright sections which exhibit the same direct proportionality.

The possibility remains that the normal polarity of upright sections of plants is partly a result of the position of the plant or section with respect to gravity, *i.e.*, that the electrical polarity is, partly, a geoelectric effect. This scheme is complicated by the return to normal polarity with time. It is not known what the effect of inverting intact plants, *e.g.*, roots or shoots, has on their electrical polarity. This problem is being investigated. The establishment of positivity of the under side of inverted sections recalls to mind the similar establishment of positivity of the under side of horizontally-placed plants (BRAUNER 13).

That the geoelectric polarity or inverted polarity is not a phenomenon confined to *Avena* sections was shown by the fact that 5-mm. inverted *Pisum* sections developed the inverted polarity. In this case, however, more time was required to establish the inversion. On first inverting, the polarity was found to be inverted a few millivolts, the maximum inverted polarity becoming established only after 3 or 4 hours. Since BRAUNER (14) showed that the geoelectric effect varied in the seed coat of various plants, depending upon the membrane structure, this is not surprising. It is likely that other

plant sections would exhibit their own peculiarities. This individuality was also observed by BRAUNER (13) for geoelectric effects in horizontally placed plants.

From the section f on electrical polarity, it was concluded that the normal, inherent electrical polarity of intact plants and cut sections of *Avena*, *Zea*, *Pisum*, and *Impatiens* was an electronegativity of the apical parts with respect to more basal parts. This polarity exists internally as well as externally, and is not directly related to growth.

### Discussion and conclusions

It has been experimentally demonstrated that the normal electrical polarity of several seedlings is an electronegativity of tip to base. Several views are held as to the mechanism of the origin of this electrical polarity.

LUND (47) has claimed that electrical polarity is the result of oxidation-reduction potentials such that usually, but not necessarily, regions of highest rates of oxidation are electropositive to other regions (or, in the thermodynamic sense of red-ox potentials, the ratios of oxidant to reductant are different in the different ends of the structure concerned). A detailed discussion of the theory is out of place at this time (LUND, 50), but certainly the theory is thrown into doubt by the fact that red-ox potentials can be measured only by indifferent electrodes, and not by non-polarizable electrodes such as used by LUND. FRANCIS (25), BEUTNER AND LOZNER (5), RAMSHORN (68), STERN (76), and DORFMAN (23, 24), have all criticized LUND's theory on this basis. STERN (76) and MARSH (56) stated that if the living membranes, to which LUND made contact with non-polarizable electrodes, acted as indifferent, metallic conducting electrodes, the P.D. measured could be the same as LUND's hypothetical oxidation-reduction chain interposed between the contacts. While it is improbable that these membranes act as metallic conductors, yet the possibility remains that they may do so.<sup>7</sup>

Bioelectric potentials, as linked with oxidative processes, can be explained by other mechanisms as well as by oxidation-reduction potentials (STERN, 76; DORFMAN, 23, 24; BEUTNER AND LOZNER, 5; FRANCIS, 25). Most of these explanations are based on the effects of oxidations on diffusion potentials or membrane potentials.

The oxidation-reduction polarity theory, moreover, is thrown into doubt by the experiments of DORFMAN (23, 24), who showed that the oxidation-reduction polarity of the frog's egg was opposite in sign to the bioelectrical polarity measured with non-polarizable electrodes.

<sup>7</sup> SCHOTT and BORSOOK (72) have shown the possibility of metallic electron conduction between enzyme centers in *E. coli*. FETCHER (dissertation, University of Chicago, 1934) demonstrated the possibility of electron conduction in membranes which are composed of conductors of the second class (LILLIE, 44).

The bioelectrical polarity of the *Avena* coleoptile is certainly not directly linked with respiration in the different regions of the coleoptile, since BONNER (6) showed that there was no distribution of respiration in this organ. Moreover, from data to be published shortly, reduced ascorbic acid (vitamin C) is found in highest concentration in the apex of the *Avena* coleoptile, the concentration decreasing basally (cystine, cystein, and glutathione are not present). It was also indicated that the reverse relation held for the distribution of oxidized ascorbic acid. This does not conform with LUND's oxidation-reduction polarity (LUND, 50), since by this theory, the tip would usually be electropositive. This is assuming, however, that the oxidation-reduction potentials of ascorbic acid could play a part in the electrical polarity. (For exceptions to this polarity rule, see LUND, 50).

With regard to the disagreement between the findings presented in this paper and those described by RAMSHORN (68), the following discussion becomes pertinent: RAMSHORN made measurements of the electrical polarity of several different seedlings and roots, and showed a parallelism between growth and electropositivity. Regions of highest growth-rate were electropositive to other regions. Temperature changed both in the same way, and applied potentials accelerated growth if the applied polarity coincided with the measured inherent polarity; and, conversely, inhibited growth if the polarities were opposed. On page 741 of his paper, RAMSHORN presents a series of curves of the gradient of electric potentials from tip to base in *Helianthus* hypocotyls after stimulation by shaking. After stimulation, the tip became electronegative to the base presumably within a few seconds. In 15 minutes the tip became electropositive, and in 75 minutes the magnitude of this positivity had diminished only a few millivolts. This roughly confirms LUND's (52) finding that the electropositivity of the apex of the Douglas fir decreases or that the tip even becomes negative on mechanical stimulation.

In the present study of the *Avena* coleoptile, reliable constancy of P.D.'s was not obtained until 90 to 110 minutes after setting up the experiment, as much care as possible being taken not to stimulate the plants during this operation. Moreover, constancy was not good unless the plants were in complete darkness; and manipulation of contacts from point to point involved considerable stimulation.

RAMSHORN's correlation between growth and electrical polarity might, in my opinion, suffer a reversal in some cases, particularly in *Avena*, if the time relations, contact manipulation, and light conditions of his experiments were reinvestigated, especially with regard to the constancy of observed P.D.'s over longer periods of time.

BARTH (3, 4) observed that apical positivity and organic polarity in the hydroids are not correlated, but that either apical or basal positivity



may be correlated with organic polarity, depending on the hydroid used. This lends no support to RAMSHORN's positivity theory; nor do REHM's (70) measurements on *Phaseolus*, in which he found the tip of the plant electro-negative to the basal regions.

CZAJA (21) says that the auxin-transport itself electrically polarizes the plant, thus roughly supporting RAMSHORN's statement that auxin changes the P.D.'s and growth rate. CZAJA, however, is largely theoretical in his consideration, and bases his assumptions on results obtained with unphysiological concentrations of auxin. In the light of unpublished experiments of my own, the effect of auxin on plant potentials is a real one, but possibly one not closely linked with the normal inherent polarity. DE HAAN (30) is also of this opinion.

Experiments on the effect of gravity on the *Avena* coleoptile P.D.'s have revealed that the electrical polarity can be changed or inverted by inverting their morphological axes. This polarity inversion is not permanent, the original polarity returning. Hence "normal" electrical polarity is not exclusively and directly caused by geoelectric potentials; but it is possible that they are contributing factors. This possibility is being examined.

The mechanism of the origin of electrical polarity might be linked with the phenomenon of polar conductance observed, e.g., by BRAUNER (14), METZNER (59), and GUHA (29). Plant tissues have been shown to exhibit a selective ionic permeability so that an electric current is conducted more easily in one direction than in the other. Investigations are under way to see if there is any relation between polar conductance, electrical polarity, and polar transport of auxin.

### Summary

1. Theories of the cause of polar transport of auxin in plants are discussed. The electrical theory has been accepted by many workers as one of the most plausible (cf. second paper, following issue of PLANT PHYSIOLOGY).

2. WENT's bipolar dye-uptake experiments on *Impatiens* cuttings, used to substantiate his electrical transport theory, are repeated and confirmed. Positively charged dyes are taken up most by bases, negatively charged dyes by apices, of *Impatiens* cuttings. This is in agreement with the fact that electrical measurements show that *Impatiens* cuttings have apical electro-negativity.

3. Intact *Avena* and *Zea* coleoptiles, *Pisum* stems, and *Impatiens* hypocotyls exhibit apical negativity when constancy of P.D. measurements is obtained. Various methods of measuring this polarity are discussed.

4. Cut sections of *Avena* and *Zea* coleoptiles, and of *Pisum* and *Vicia* stems exhibit the same polarity found in *Impatiens* cuttings, i.e., apical negativity. Time is required to establish this polarity. The P.D.'s of sections are directly proportional to the length of the sections.

5. The internal electrical polarity of the *Avena* coleoptile is the same as the external.

6. Inverting sections inverts their electrical polarity, *i.e.*, the morphological apices become electropositive to the bases. This inverted polarity disappears with time. It is proportional to the length of the sections as in the case of upright sections; and the time of disappearance of the inverted polarity is proportional to the length of the sections.

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# EFFECT OF ETHYLENE AND CERTAIN METABOLIC GASES UPON RESPIRATION AND RIPENING OF PEARS BEFORE AND AFTER COLD STORAGE

ELMER HANSEN AND HENRY HARTMAN

(WITH FIVE FIGURES)

## Introduction

Previous work by the writers (6) has shown that the ripening rate of newly picked pears can be markedly stimulated by ethylene or gases of similar properties naturally evolved, but fruit treated after being held for prolonged periods of cold storage is not similarly affected. As a tentative explanation of these differences, it was suggested that the effects of ethylene are confined to a pre-ripening period, and later ripening changes apparently are unaffected by the presence of the gas in the atmosphere surrounding the fruit. These observations are in agreement with those previously reported by KIDD and WEST (1) for apples.

The present investigation was undertaken to further study the effects of naturally occurring ethylene upon the ripening processes in pears and to more definitely determine if there is a stage in the life of the fruit when treatment with this gas is most effective.

## Material and Methods

The varieties of fruits used in these experiments were Bartlett, Bosc, Comice, and Anjou pears. With the exception of the first mentioned variety, these were grown in the Medford (Oregon) district. Fruit intended for treatment immediately after picking was gathered at approximately ten-day intervals, beginning a month or more prior to and extending considerably beyond the regular commercial harvest season. Fruit intended for storage studies was picked at one date when the proper picking stage for the variety had been reached.

Immediately after picking or removal from storage, a pressure test was taken of each lot, which was then subdivided into two duplicate lots of 10 to 15 pears each. These were weighed, placed in five-gallon glass jars provided with air-tight lids, fitted with inlet and outlet tubes.

To prevent any accumulation of gases that might be effective in stimulating ripening of fruit within the containers, the untreated lots were provided with constant aeration in the following manner: An air line with an inlet to a fresh air supply was installed in the ripening room. The jars containing the untreated samples were connected to outlets on the air line and continuous ventilation was provided by means of a vacuum pump operating



on the reverse end. The incoming air before passing over the fruit was brought to room temperature and conditioned for humidity by passing through a five-foot length of pipe and finally bubbling through one-half inch of water maintained at room temperature.

The treated fruit was supported in the jars on a false bottom, permitting the use of a 15 per cent. KOH solution beneath to absorb the carbon dioxide evolved and to maintain a humidity comparable to that in the ventilated jars. Atmospheric concentration of oxygen was maintained by the addition of pure oxygen from a constant water level siphon. In those lots treated with ethylene artificially applied, the concentration of gas used, in all cases, was one part by volume to 1000 parts of air. In the tests with naturally evolved gases, three or four ripe pears were enclosed in the jar together with the fruit to be ripened. The fruit was kept in the containers continuously during the time of treatment except for a period of one to two hours daily when removed to determine the rate of respiration. In the cases where ethylene was applied, the gas was replenished after each removal of the fruit from the jars. The KOH solutions were renewed often enough to assure efficient absorption of carbon dioxide.

Respiration determinations were conducted in a room maintained at a temperature of 65° F. Although thermograph records showed in some cases a variation of two to three degrees, this was not considered to have materially affected the differences in rate of respiration observed between treated and untreated lots, since both were always subject to the same temperature changes. The method of HARDING, MANEY, and PLAGGE (7), with slight modifications, was used for carbon dioxide determinations. The carbon dioxide was absorbed in 0.15 N barium hydroxide and titrated directly with the N/10 hydrochloric acid, using thymolphthalein as indicator. Determinations were made daily for a period of seven to nine days with the storage series, but with newly-picked fruit this period was considerably extended because of the longer period of time required for ripening. At the end of the experiment, loss in weight for each lot was determined.

The rate of ripening of treated and untreated lots was determined by the comparative softness of the flesh as indicated by the Oregon pressure tester, and by the number of days required for the fruit to attain an edible condition. With the newly picked fruit, where carbon dioxide determinations were extended in some cases over a period of thirty days or more, the number of days required for ripening only was used as an index of ripening rate. With the storage series, a pressure test was made on one-half of both treated and untreated lots at the end of nine days and the remainder kept to determine the number of days required for ripening. If the fruit was ripe before the expiration of nine days, the entire sample was used for a pressure test.

### Experimental results

#### I. EFFECT OF NATURALLY EVOLVED GASES AND OF ETHYLENE ARTIFICIALLY APPLIED UPON RESPIRATION AND RIPENING OF NEWLY- PICKED FRUIT

SELECTION OF SAMPLES AND TREATMENT.—The samples of each variety used in the experiments with newly-picked fruit were selected from single trees and represented the average size of the fruit at the time of gathering. The first two pickings of Bartlett were made approximately two months prior to the commercial harvest season, but because of the small size and undeveloped condition of these fruits no treated lots were included. The data are presented, however, to show the trend in respiration at this early period in the season. A sample of the third picking was treated with ethylene gas, since no ripe pears were available at that time, but all subsequent lots of newly-picked fruit to be treated were inclosed with ripe pears as indicated in the general methods.

TABLE I

EFFECT OF RIPE FRUIT UPON THE RIPENING OF NEWLY PICKED BARTLETT, ANJOU, AND COMICE PEARS

VARIETY	DATE PICKED	DAYS REQUIRED FOR RIPENING		DIFFERENCE IN DAYS REQUIRED FOR RIPENING
		TREATED	UNTREATED	
Bartlett	{ 7-22*	8	20	12
	{ 8-20	8	14	6
	{ 9-4	7	12	5
	{ 9-17	6	8	2
Comice	{ 8-16	20	†	
	{ 8-30	15	†	
	{ 9-20	11	30	19
	{ 10-8	8	15	7
Anjou	{ 8-16	18	†	
	{ 8-30	12	†	
	{ 9-16	12	35	23
	{ 10-8	8	15	7

\* Treated with ethylene.

† Failed to ripen in 30 days.

#### EFFECT UPON RATE OF RIPENING

That the gas produced by ripe pears does have a very pronounced effect upon the ripening of newly-picked fruit is evident from these studies. As shown in table I, the treated lots, subjected to the influence of several ripe pears in closed containers, ripened in comparatively short periods of time.

Duplicate lots, however, that were not subjected to the influence of ripe fruit, but kept under constant ventilation with fresh air, either failed to ripen or were markedly delayed beyond the time required for ripening of the treated fruit.

But it is clearly evident from the results obtained that while the period required for ripening can be significantly reduced by these naturally occurring emanations, the magnitude of the decrease obtained depends upon the degree of maturity of the fruit at the time of picking. Thus, with an early picking of immature Bartlett, there was a difference of 12 days between the ripening time of treated and untreated lots. With post-mature fruit, however, the ripening period of the treated lot was only two days less than that of the untreated. Similar results were observed with Anjou and Comice pears. These varieties respond to ethylene over a longer period, however, than do Bartlett. Reference to table I shows that while both of these varieties were markedly affected in rate of ripening by the emanations from ripe pears, the greatest response was obtained in those lots picked early in the season. Thus, the treated fruit of the first pickings ripened in 15 to 18 days, while the untreated lots failed to ripen in 30 days and the condition of the fruit at that time was such as to indicate that ripening would be delayed indefinitely. Later pickings showed decreasingly smaller differences in the ripening time between treated and untreated lots. Post-mature samples, however, picked three weeks after the commercial season still showed seven days' difference between treated and untreated lots.

#### EFFECT UPON RESPIRATION

The increase in the rate of ripening of the treated fruit was accompanied by a marked and rapid increase in respiration (figs. 1, 2, 3). The gases evolved by ripe pears inclosed with the unripened fruit had the same effect in stimulating respiration as did ethylene added to the containers. In the

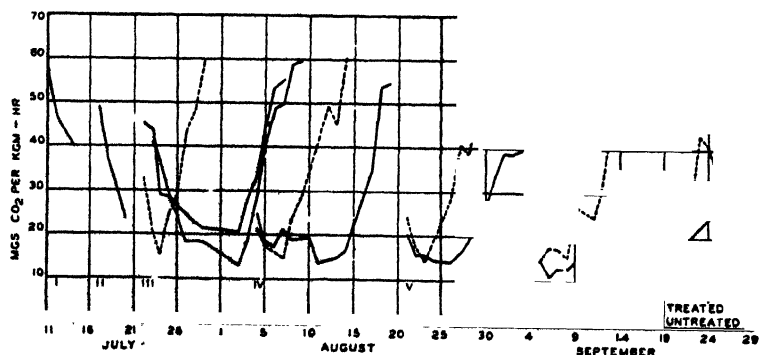


FIG. 1. Effect of gases produced by ripe pears upon the respiration of Bartlett pears picked at different stages of maturity.

untreated lots no similar increase in respiration occurred until after a delayed period of time. The length of this delay, however, was considerably longer with early- than with late-picked fruit, and appeared, therefore, to be associated with the maturity of the fruit at the time of removal from the

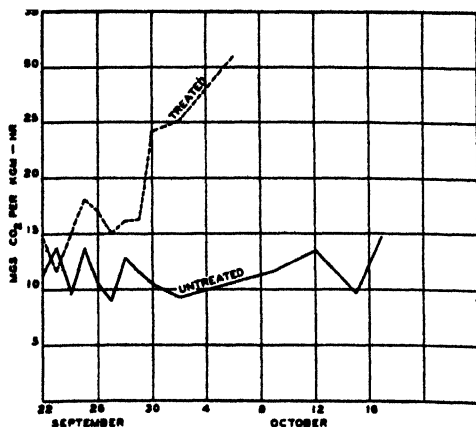


FIG. 2. Effect of gases evolved by ripe pears upon the respiration of Anjou pears treated immediately after picking.

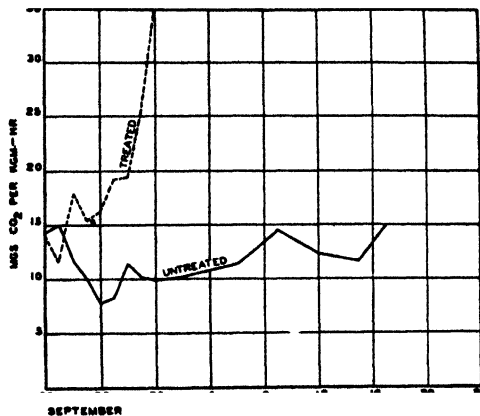


FIG. 3. Effect of gases evolved by ripe pears upon the respiration of Comice pears treated immediately after picking.

tree. Thus in lot III, an early picking of Bartlett, a period of 12 days elapsed in the case of the untreated fruit before an increase in respiration occurred. With fruit gathered at later dates, however, the period between the time of picking and the increase in respiration accompanying ripening became progressively shorter and shorter until with the post-mature lot, the respiratory rate rose rapidly and almost immediately after picking.

It appears, therefore, that fruit in advanced stages of maturity is already at a stage where respiration is tending toward a high rate. Naturally with these pears, little effect from treatments to stimulate respiration can be obtained. Fruit in earlier stages of maturity, however, is either at a stage of low respiratory activity or is tending to drift from a high to a low rate. With these fruits, where the period of low respiratory activity is considerably extended, treatments to stimulate respiration can be expected to be most effective.

As long as the fruit remains attached to the tree, the increase in respiration appears to take place very slowly, and consequently it is considerably beyond the commercial season before the period is reached when pears will no longer be affected by ethylene or naturally occurring gases. Fruit picked during the commercial season was still respiring at a comparatively low rate at the time of picking and showed a marked effect from treatment.

That the effect obtained from ethylene treatment bears a relationship to the maturity of the fruit has also been observed by other workers. WORK (16), for instance, obtained the greatest effect from treatment of tomatoes picked 30 to 40 days from blooming. DAVIS and HURCH (1) observed that the stimulative effect of ethylene on the respiration of Japanese persimmons declined as the fruit ripened, especially in the last stages and in the stored fruit. With bananas, HARTSHORN (8), using acteylene, found that treatment appeared to be most effective in shortening the initial period of low activity in respiration and consequently the greatest differences were found in those lots in which this period is of greatest duration. None of these workers, however, have associated these differences in responses to ethylene treatment with natural progressive changes in respiration occurring in the fruit during maturation.

KIDD and WEST (9), working with apples, first designated the period of rapid increase in respiration as the "climacteric" and have observed (11) that while the emanations from ripe apples have the property of hastening the onset of the climacteric, such volatile products were ineffective during the post-climacteric period when softening of the flesh and other outwardly indicated ripening processes occurred. With pawpaws, however, WARDLAW and LEONARD (15) have reported that coloring, softening, and final maturation of the fruit are coincident with the climacteric rise. MAGNESS and BALLARD (13) have similarly found that the respiration of Bartlett pears increases rapidly as the fruit ripens. The respiration curves of these writers do not show such an extended low level, however, as has been observed in these experiments. This can probably be explained on the basis that in their experiments a period of 3 to 5 days elapsed between the time of picking and the initial run, and during this delay the fruit had probably been subjected to conditions favorable for ripening. It was observed, however, that early-

picked fruit had a lower initial rate of respiration than late-picked samples, and also that the speed with which respiration rate increased was less rapid in the early-picked than in the late-picked fruit.

#### COMPARATIVE EVOLUTION OF ETHYLENE AT DIFFERENT PERIODS

In relation to the differences in results obtained with fruit picked at various stages of maturation, it will be interesting to know at what period the greatest production of volatile substances affecting ripening occurs. KIDD and WEST (10) have stated that the evolution of the effective gas is associated with the climacteric in apples and bananas, but later observations (2, 6, 12) have indicated that the pre-climacteric fruit also shows indications of ethylene production. If ethylene is a normal respiratory product in some fruits and plant tissues and shows concomitant increases or decreases with carbon dioxide production, then there are two stages in the life of the pears when evolution of ethylene should be greatest. The first would be in the premature fruit when the rate of respiration is high and the second during ripening when  $\text{CO}_2$  production is also maintained at a high level. That there is not a comparable evolution of ethylene at these two periods, however, was indicated by these experiments.

While the presence of ethylene, as indicated by leaf epinasty, could be detected in the premature fruit, the epinastic effect produced by an equal weight of fruit respiring at the same rate, but during the climacteric was strikingly more pronounced. This observation seemed to hold true not only for Bartlett pears, but for the other varieties studied as well. The indications are, therefore, that the evolution of ethylene during the life of the fruit is greatest during the period shortly preceding and including ripening, and is not necessarily concomitant with carbon dioxide production at other periods. Definite proof of this statement, however, must await the development of an accurate method for the determination of small amounts of ethylene.

Since even immature fruit produced a small amount of ethylene as indicated by leaf epinasty, the indications are that no lot of fruit under observation was ever entirely free from the effects of the gas produced within the tissues. In view of this, it appears that the determination of the physiological behavior of fruit entirely isolated from the presence of ethylene will be extremely difficult. The results of these studies indicate, however, that this gas naturally evolved is an important factor to be considered in the respiration and ripening of fruit. The fact alone that there is apparently a very marked increase in the production of ethylene at the beginning of the climacteric would suggest this possibility. Furthermore, the fact that respiration was retarded and ripening was delayed in those lots kept free from an accumulation of any gases by constant aeration, whereas respiration

was increased and ripening hastened in those lots where the gases were allowed to accumulate, strongly indicates that the gas naturally evolved by the fruit, while not necessarily indispensable to ripening processes, does markedly hasten the period when ripening occurs.

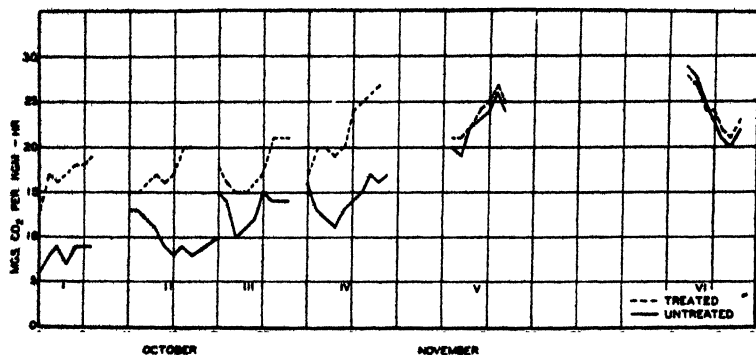


FIG. 4. Effect of ethylene upon the respiration of Anjou pears treated at 65° F. after being held at 37° F. for various periods.

## II. HOW LONG AFTER PICKING CAN AN EFFECT FROM ETHYLENE BE OBTAINED?

**METHODS.**—Since pears are comparatively short lived at high temperatures, it was difficult to determine at just what stage after picking the fruit ceased to respond to ethylene, although the data obtained indicated that treatment was ineffective after the rapid rise in respiration had been nearly completed. In order to follow the changes in response to ethylene treatment after picking more closely, it was decided to use a variety that would show a response over the greatest possible period of time and to further extend this period by the use of cold storage. For this reason, the Anjou pear, a long-lived variety which keeps well under cold storage conditions, was selected. The fruit was picked at commercial maturity from a single tree, and at the time of gathering was in the pre-climacteric stage. Immediately after gathering, two lots were carefully selected for uniformity of size, packed in oil wraps, and stored at 31° and 37° F. respectively. At approximately 12-day intervals, a sample consisting of 30 pears was withdrawn from each lot, taken to the 65° F. ripening room, and treated as indicated in the general methods. As a comparison to the Anjou pear, some studies were also made on Bosc and Comice, which are shorter lived varieties. The method of handling was the same as outlined with the exception that the 37 degree storage lots were not included.

### EFFECT UPON RESPIRATION

The period during which the Anjou pears were under observation extended from September 16 to December 12. During this time respiration

data were obtained on six lots removed from storage at consecutive dates. The curves in figure 4, therefore, show, over a fairly long period, the general trends in respiration during ripening of both treated and untreated lots.

That the effect of ethylene upon the rate of respiration steadily decreases after storage is clearly indicated by the results obtained. References to figure 4 show that while there is a very pronounced difference in the rate of

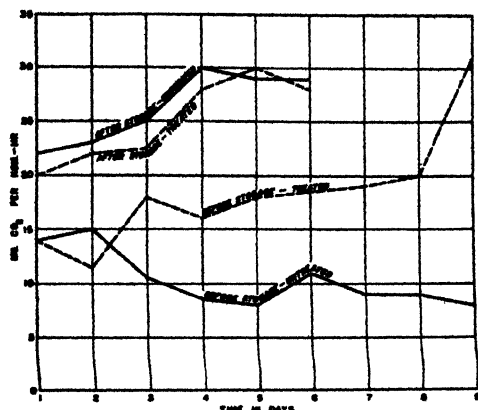


FIG. 5. Effect of ethylene upon the respiration of Comice pears treated before storage and after 6 weeks' storage at 31° F.

respiration between treated and untreated lots after removal from storage early in the season, the magnitude of this difference gradually decreases during a period of two to three months until a point is reached where respiration of the untreated fruit is maintained at as high a level as that of the treated. This period when respiration is no longer affected by ethylene treatment was reached in nine weeks by pears stored at 37° F., while approximately one month longer was required for the pears stored at 31° F. With Comice (fig. 5) no response could be obtained after six weeks, while Bosc were unaffected after only two weeks of cold storage.

Further reference to figure 4 shows that the increase in the rate of respiration, which can be obtained from ethylene over that of untreated lots, is directly correlated with the initial rate or level at which the fruit is respiring at the time of treatment. When this level is low a large increase can be obtained, but when the level is high the difference is correspondingly reduced. Thus, the initial rate of respiration of the first lots removed was at a comparatively low level. The greatest increase was noted in these lots. On subsequent dates, however, there was a gradual increase in the level of respiration of the fruit after removal from storage which was accompanied by a corresponding decrease in effect from ethylene treatment. This trend continued until a point was reached where the fruit, after removal to ripening temperature, was respiring at a high rate, and with these lots no further



increase above the already high rate could be obtained. It appears, therefore, that the rate at which the fruit can carry on respiration is limited to a definite maximum, over and above which no increase can be obtained with ethylene treatment. Furthermore, it appears that there is a gradual drift in respiration toward this high level whether the fruit is held at high temperatures or in cold storage.

#### EFFECT UPON RATE OF RIPENING

Evidence that the effect of ethylene upon the rate of ripening steadily decreases after storage is shown by the differences in ripening rate between treated and untreated lots removed at the different periods. As shown in table II, of the fruit stored at 37° F. for two weeks, the untreated lot required 17 days to ripen, while the corresponding treated lot ripened in 10 days. After a period of 9 weeks, however, the untreated fruit, upon removal from storage, ripened as rapidly as the treated fruit. Similar results were obtained with the 31° F. series, but with these pears, there was still a slight difference in the number of days required for ripening of treated and untreated fruit after 12 weeks. With Comice, after six weeks' storage, the untreated lots ripened as rapidly as the treated, while Bosc showed no response after two weeks of cold storage.

It might be expected that this observed decrease in the time required for ripening could be due to progress in ripening of the fruit while in storage. On the contrary, the indications were that ripening processes, as outwardly manifested at least, had preceded very little during this period. From all appearances, the fruit which showed no response to ethylene was still nearly as much in an unripened condition as those samples which were markedly affected by ethylene treatment earlier in the season. At the time

TABLE II

EFFECT OF ETHYLENE UPON RESPIRATION AND RIPENING RATE OF ANJOU PEARS AT 65° AFTER REMOVAL FROM STORAGE AT 37° F.

NO. DAYS OF STORAGE	PRESSURE TEST AT TIME OF REMOVAL	INITIAL RATE OF RESPIRATION MG. CO <sub>2</sub> /KG./HR.		PRESSURE TEST AFTER 9 DAYS' TREATMENT		DAYS TO RIPEN	
		TREATED	UNTREATED	TREATED	UNTREATED	TREATED	UNTREATED
12	19.0	13.63	6.74	3.1	13.9	10	17
24	18.7	15.94	13.53	2.5	8.1	9	15
36	17.8	18.74	14.74	2.5	6.1	8	12
48	17.3	17.09	16.71	2.5	3.7	7	10
60	17.0	20.50	20.00	2.0	2.3	6	6
90	8.5	28.00	29.00	2.0	2.0	5	5

of picking the pressure test of the fruit, an indication of firmness or flesh, was 19 pounds. The pressure test of the first lot failing to respond to treatment was still 17 pounds, indicating that very little softening of the fruit had occurred during storage. The change in the fruit, then, that is correlated with the decrease in effect from ethylene, must be accounted for on another basis.

In table II are shown the trends in initial rates of respiration, the number of days required for ripening, and the pressure tests before and after ripening for both treated and untreated lots of the 37-degree series. As previously pointed out, the initial rate of respiration of the untreated lots, upon removal from cold storage, shows a progressive increase. The number of days required for ripening and the pressure test taken nine days after removal from storage show a steady decline in the untreated lots. The pressure test of the unripened fruit before treatment shows very little decrease for nine weeks, but subsequently a very sudden and marked decline occurs. The striking fact brought out is the correlation existing between the initial rate of respiration and the ripening rate of the fruit. Thus, the period when the fruit has nearly attained its maximum initial rate of respiration upon removal from storage, almost exactly corresponds to the period when the fruit ceases to respond to ethylene treatment. This would indicate that, with these storage pears, the effects of ethylene were confined to the period during which the maximum level in respiration was being attained. Furthermore, indications are that the ripening processes including softening of the flesh, occurring after the peak in respiration had been reached, were not affected by the addition of this gas to the ripening chambers.

That the respiration of pears after delayed periods of cold storage starts off at a markedly higher rate than does that of similar fruit before storage, has been previously shown by MAGNESS and BALLARD (13). These writers also found that an increase in the respiration of Bartlett pears occurred while the fruit was held at 37° F., but no increase was noted with similar fruit held at 31° F. GERHARDT and EZELL (4), however, have found that Comice pears show a consistent increase in respiration during storage at 31° F.

#### EFFECT OF ETHYLENE UPON PEARS HELD AT COLD STORAGE TEMPERATURES

The results of preliminary investigations, the data of which are not herein included, indicate that ethylene has very little effect upon ripening or respiration of pears held at cold storage temperatures. Bosc, Comice, and Anjou pears treated for periods of from one to three months at 31° and 37° F. showed no apparent increase in respiration or rate of ripening over that of the untreated lots. The indications are, therefore, that the slow progress of the climacteric at low temperatures is not influenced by an increase in the concentration of ethylene over that naturally produced within the fruit tissue.

These results are in agreement with those of KIDD and WEST (12) who have observed that the respiration of apples was not increased at cold temperatures upon exposure to the vapors produced by ripe fruit. DUSTMAN (3), however, has reported increased rate of ripening of Ben Davis, Rome Beauty, and Stayman apples treated with ethylene for one month at cold storage temperatures.

### Discussion

The time of ripening in relation to the occurrence of the climacteric is obviously different with pears held under cold storage conditions than with fruit held at higher temperatures. It will be recalled that with newly-picked pears, ripening symptoms were first apparent with the start of the climacteric; and before the peak of respiration had been reached, the fruit had become fully ripe. With storage fruit, however, while apparently the climacteric occurred at cold temperatures, ripening was delayed until the post-climacteric period or until the fruit was removed to temperatures favorable for ripening. Since the factors associated with the climacteric rise are not clearly understood at the present time, an explanation for the differences observed is not readily apparent. It is clearly indicated, however, that fruit picked in a stage of low respiratory activity tends to drift toward a high maximum rate whether held at high or low temperatures, and while the increase in respiratory activity may occur independently of the ripening of the fruit, activation of the ripening processes appear to be closely associated, at least, with the incidence of this period.

The results obtained with both pre- and post-storage pears indicate that the effects of ethylene are definitely confined to a period preceding the stage in the life of the fruit when the maximum level in respiration has been reached. With fruit treated immediately after picking, the greatest response from treatment was obtained with those fruits having the greatest length of time elapsing before the climacteric, while the least response was obtained in those cases when at the time of picking this rise in respiration had started or was well under way. The results obtained with fruit treated after delayed periods of storage clearly show that ethylene treatment is effective only during the period of ascending respiratory activity, and the magnitude of the response obtained bears a direct relationship to the increase in respiration that has naturally taken place prior to treatment.

The marked differences observed in the periods of time during which long- and short-lived varieties of pears respond to ethylene treatment can be cited as further evidence that ethylene treatment is effective during only a definite period in the life of the fruit. With the Anjou pears, a long-lived variety, over 12 weeks of storage at 31° F. had elapsed before the fruit ceased to respond to treatment. With the Bosc pear, a short-lived variety,

no effect from ethylene could be obtained after two weeks of storage. An intermediate-lived variety, the Comice, showed no response after six weeks of storage. Furthermore, Anjou pears stored at 31° F. responded to ethylene treatment over a period of four weeks longer than did pears of the same variety held at 37° F. It appears, therefore, that with all the varieties of pears studied, there is a gradual drift toward a period of a maximum level in respiration, and when this stage in the life of the fruit has been reached, ethylene treatment is no longer effective. While external factors such as changes in temperature do retard or accelerate the rate at which this increase in respiration takes place, the general trend followed appears to be controlled primarily by internal factors inherent within the fruit.

Since a trend in respiration during maturation similar to that shown by pears has been observed to occur in a number of other fruits (5, 9, 14, 16), it may possibly follow that these fruits would similarly respond to ethylene treatment only during a definite life period. This being true, the wide diversity of results that have been obtained from the use of ethylene as a ripening agent can be at least partly explained on this basis.

### Summary

1. The emanations from ripe pears have been found to increase the rate of respiration and ripening of newly-picked Bartlett, Comice, and Anjou pears. The greatest effect from treatment was obtained with fruit picked early in the season, and the least effect with fruit picked at post-mature stages.

2. The production of ethylene, as indicated by leaf epinasty, appears to increase during the period of ascending respiratory activity accompanying ripening of the fruit.

3. No increase in respiration or ripening could be obtained from ethylene treatment of fruit which had been held in cold storage for certain periods. Long-lived varieties responded over a greater period than did short-lived varieties. Pears held at 31° F. responded for a greater length of time than did similar fruit held at 37° F.

4. The decrease in effect from ethylene treatment appears to be associated with a natural increase in the respiratory activity of the fruit after picking.

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# EXCHANGE OF ELECTROLYTES BETWEEN ROOTS AND ACID SOLUTIONS<sup>1</sup>

RUTH BEALL

(WITH FIVE FIGURES)

## Introduction

This paper presents, in abbreviated form, some results of a study of changes in electrical conductance and in hydrogen-ion concentration of dilute acid solutions exposed to the action of seedling roots of *Lupinus albus* L. Effects of these solutions on the plants are also described.

## Materials and methods

Three inorganic acids ( $\text{HCl}$ ,  $\text{H}_2\text{SO}_4$ , and  $\text{HNO}_3$ ) and three organic acids (formic, acetic, and propionic) were tested separately, each at three concentrations, 0.00002 N, 0.00008 N, and 0.00016 N. Expressed as molar fractions these concentration values are M/25,000, M/12,500, and M/6250 for  $\text{HCl}$ ,  $\text{HNO}_3$ , and the organic acids; and they are M/50,000, M/25,000, and M/12,500 for  $\text{H}_2\text{SO}_4$ . The water used was redistilled from Pyrex glass and had a conductance of approximately 2 millionths reciprocal ohm. For control cultures DOAK's (11) modification of HARTWELL and PEMBER's nutrient solution was used, as well as  $\text{H}_2\text{O}$ .

Seeds of white lupine were germinated in finely chopped sphagnum and the resulting seedlings were introduced into the test solutions when primary roots and hypocotyls were about 6 cm. and 3 cm. long, the cotyledons being still within the seed coats. After rinsing, four seedlings were introduced into each culture jar. The jars used were 500-ml. Pyrex beakers, each containing 500 ml. of liquid. The seedlings were supported by means of cotton in holes in the jar lids, which were perforated Petri-dish covers of Pyrex glass (2). The roots were wholly submerged and the hypocotyls were entirely above the liquid surface. Thus nearly the same area of root surface was exposed to the solution in every case.

These experiments were carried out in a dark-room at the Botanical Laboratory of the University of Pennsylvania. For each set of solutions there were two series of experiments, the temperature for one series being about 22° C. for the mineral acids, and about 23° C. for the organic acids; that for the other series being about 28° C. Temperature fluctuated somewhat in each case, within plus or minus 2°.

<sup>1</sup> This experimentation was carried out with the financial aid of a Lydia T. Morris Fellowship of the Morris Arboretum of the University of Pennsylvania.

Changes in ionic concentration of the solutions surrounding the roots were followed by means of daily measurements of electrical resistance, from which were derived the corresponding specific conductance values, computed for a temperature of 18° C. Daily measurements of hydrogen-ion concentration were made by means of the quinhydrone-titration method, with McILVAINE's buffers (4). Since the temperature coefficient of hydrogen-ion concentration is so small as to be negligible in such a study as this (7), the pH values were not corrected for temperature. Daily observations concerning the appearance of the plants were also made, particularly with regard to development and visible injury.

In the following subsections, changes in the media will be presented first, distilled water and the several acid solutions being considered in order, and then attention will be turned to records of growth and of injury to the plants.

## Experimentation

### CHANGES IN THE MEDIA

**DISTILLED WATER.**—Figure 1 presents graphically average daily con-

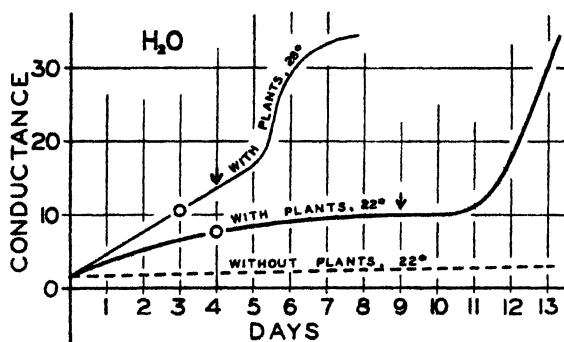


FIG. 1. Conductance (millionths recip. ohm) of H<sub>2</sub>O with and without seedlings. Circles mark end of rapid initial increase in pH; arrows mark onset of visible root injury.

ductance values for H<sub>2</sub>O with and without seedlings at 22° C., and for H<sub>2</sub>O with seedlings at 28° C. Since no considerable conductance increase occurred without plants, it may be inferred that the increases shown by water with plants were due to outward movement of material from the roots. There was continuous exosmosis of electrolytes, in excess of any absorption that may have occurred. Net exosmosis, which was at first relatively slow, became markedly accelerated about the fifth day at the higher temperature and about the eleventh day at the lower temperature. It was at all times more rapid at the higher temperature than at the lower. At the lower temperature the pH value of H<sub>2</sub>O with seedlings increased from 5.4 to 5.7 in

the first day, and then to 6.1 in the following three days, after which it remained practically unchanged. At the higher temperature the changes were very similar, but more rapid, from 5.0 to 5.5 in one day, and to 6.1 in three days. This may indicate either that there was some absorption of hydrogen ion, the effect of which on conductance was overbalanced by relatively greater exosmosis of electrolytes, or that exosmosis acted to reduce the hydrogen-ion concentration of the surrounding liquid.

MINERAL ACID SOLUTIONS.—The manner in which conductance of the mineral acid solutions in contact with seedling roots first decreased and subsequently increased is shown by the graphs of figures 2 to 4. Each graph

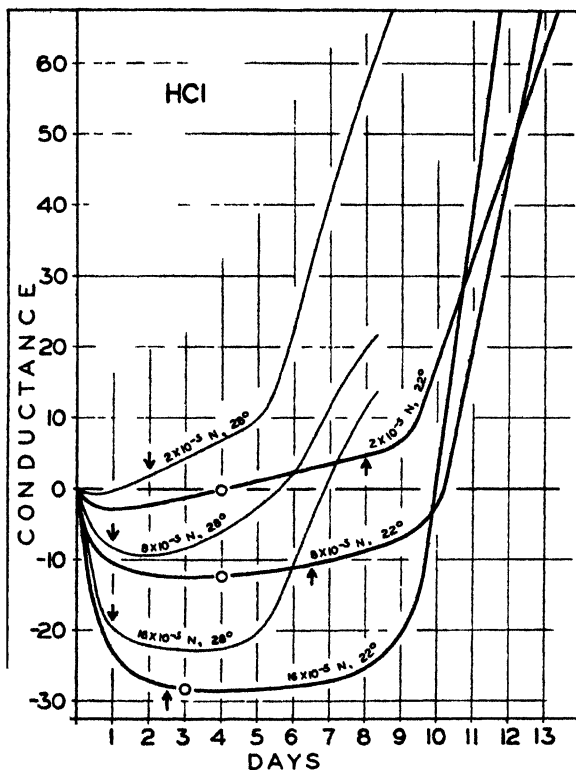


FIG. 2. Conductance change (millionths recip. ohm) in HCl solutions with seedlings. Circles mark end of rapid initial increase in pH; arrows mark onset of visible root injury.

starts at zero on the scale of ordinates (which, in each case, represents the initial conductance of the solution in question) and conductance decreases are plotted as negative while increases are plotted as positive. The three average initial conductances, expressed as reciprocal ohms  $\times 10^{-6}$ , for each of the three concentrations of each acid were as follows: HCl, 8.9, 26.8, 52.8; H<sub>2</sub>SO<sub>4</sub>, 8.8, 26.9, 56.2; HNO<sub>3</sub>, 8.5, 31.3, 64.5.



Some special measurements made at the end of the first hour showed, in many instances, a slight initial increase in conductance, relatively greater as the original concentration of the solution was higher, which suggests that there may have been an immediate exosmosis of electrolytes from the roots. This detail is not shown on the graphs; it was not seriously studied and warrants no more than mention here.

Each of the graphs shows an initial decrease in conductance, which was very slight and of very short duration for the solutions of lowest concentration, but progressively more pronounced and of longer duration as concentration was higher. Considering the two graphs for each concentration together, it may be said that the period of conductance decrease was shortest

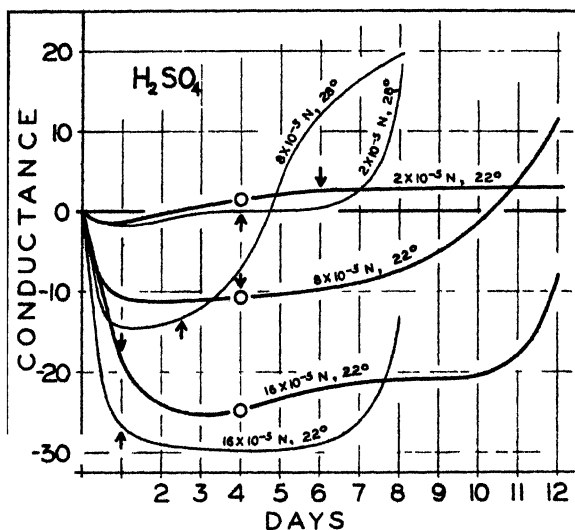


FIG. 3. Conductance change (millionths recip. ohm) in H<sub>2</sub>SO<sub>4</sub> solutions with seedlings. Circles mark end of rapid initial increase in pH; arrows mark onset of viable root injury.

(about 1 day or less) for the lowest concentration, longer (about 2 to 3 days) for the intermediate concentration, and longest (about 3 to 4 days) for the highest concentration. Similarly, the average amounts of conductance decrease were approximately as follows, the unit being  $1 \times 10^{-6}$  reciprocal ohm: for the lowest concentration, 2 units (all three acids); for the intermediate concentration, 11 units (HCl), 13 units (H<sub>2</sub>SO<sub>4</sub>), and 17 units (HNO<sub>3</sub>); for the highest concentration, 26 units (HCl), 28 units (H<sub>2</sub>SO<sub>4</sub>), and 41 units (HNO<sub>3</sub>). It is notable that HNO<sub>3</sub> showed greater conductance decrease at the two higher concentrations than was shown by either of the other mineral acids at these concentrations.

As to the temperature relations of the amount of conductance decrease, the available data permit no satisfactory generalization for all three mineral acids. From the graphs of figure 2 it is clear that the amount of conductance decrease for solutions of HCl was regularly somewhat greater at the lower than at the higher temperature. For the lowest and highest concentrations of  $\text{HNO}_3$  (fig. 4) the same temperature relation apparently holds, but for the intermediate concentration of  $\text{HNO}_3$ , and for all tested concen-

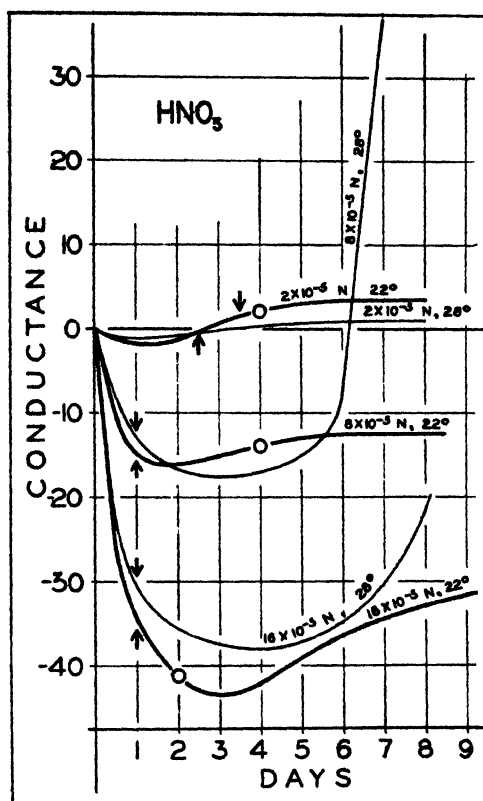


FIG. 4. Conductance change (millionths recip. ohm) in  $\text{HNO}_3$  solutions with seedlings. Circles mark end of rapid initial increase in pH; arrows mark onset of visible root injury.

trations of  $\text{H}_2\text{SO}_4$  (fig. 3) conductance decrease was more or less markedly greater at the higher than at the lower temperature.

When conductance decrease was relatively large in amount its rate is seen to have been rapid at first and then progressively more gradual. For the intermediate and highest concentrations the initial period of decrease was followed by a more or less prolonged period during which conductance was nearly maintained. This period was longer for the lower temperature

than for the higher one. After conductance began to increase, its rate of change sometimes remained low to the end of the experimental period, but in many cases (notably all the graphs of figure 2) its rate was soon rapidly accelerated, so that the final portions of the graphs ascend very steeply.

As to changes in hydrogen-ion concentration, the pH values of all tested solutions—and that of  $H_2O$  also—increased from the beginning of the experimental period onward. This increase was most rapid during the first day and became progressively slower till a value of about 6.0 was reached, when hydrogen-ion equilibrium with the roots was apparently established. Eventually the solutions became slightly more basic. The initial pH values of the solutions, corresponding to the initial conductances given above, were as follows:  $HCl$ , 4.6, 3.9, 3.6;  $H_2SO_4$ , 4.5, 3.9, 3.6; and  $HNO_3$ , 4.5, 3.8, 3.5. After one day the following values were reached:  $HCl$ , 4.8, 4.6, 5.3;  $H_2SO_4$ , 5.1, 4.6, 5.5; and  $HNO_3$ , 4.9, 5.1, 5.6. The time required, at the lower temperature, for each medium to attain a pH value of about 6.0 is shown by the position of the circle on the corresponding graph of figures 1 to 4. That time was in general somewhat shorter for the solutions of highest concentration than for the other solutions and for  $H_2O$ . The two lower concentrations of the three acids were essentially alike, reaching this value in four days; for the highest concentration the change was somewhat more rapid in  $HNO_3$  (2 days) than in  $HCl$  (3 days) or  $H_2SO_4$  (4 days).

ORGANIC ACID SOLUTIONS.—Conductance changes shown by the three organic acids in contact with seedlings are set forth by the graphs of figure 5, which are plotted like those of figures 2 to 4. For each of the three concentrations of each acid, the initial conductance (each plotted as zero on the ordinate scale) was as follows:  $HCOOH$ , 6.2, 16.2, 29.3;  $CH_3COOH$ , 5.5, 10.6, 16.3;  $C_2H_5COOH$ , 4.2, 9.6, 14.1. For each solution the two temperatures tested showed no essential differences in conductance change; the graphs of figure 5 represent the average values for the two temperatures. As in the case of the mineral acids, conductance measurements made within the first day of the experimental period indicated in some instances a very slight immediate increase in conductance, of very short duration, which is not shown on the graphs.

These organic acid solutions showed an initial decrease in conductance, similar to that shown by the mineral acid solutions, but less pronounced. For the lowest concentration of each acid this decrease was of one day's duration or less. It was scarcely noticeable in the case of the most dilute solution of propionic acid, only very slight in the weakest acetic acid solution, and somewhat more pronounced in the lowest concentration of formic acid. In all these cases conductance subsequently increased gradually for about three days, after which it remained approximately unchanged. For

solutions of higher concentration the initial conductance decrease lasted somewhat longer, from one to three days, after which there was, in most cases, some increase, but this was never sufficient to raise the conductance of the solution above its original value. In general, conductance remained unchanged after five days. As was shown for the inorganic acid solutions, initial decrease was greater in amount as solution concentration was greater, the amounts of decrease (in conductance units) being as follows:  $\text{HCOOH}$ , 2, 11, 20;  $\text{CH}_3\text{COOH}$ , 1, 4, 8;  $\text{C}_2\text{H}_5\text{COOH}$ , 0.1, 3, 6.

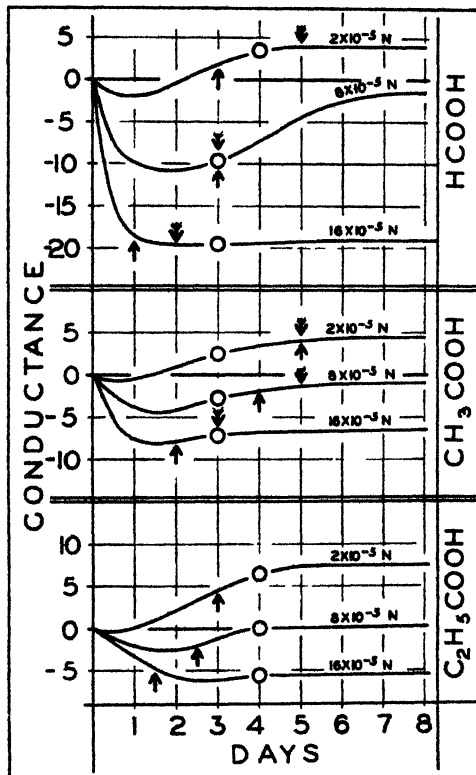


FIG. 5. Conductance change (millionths recip. ohm) in organic acid solutions. Circles mark end of rapid initial increase in pH; winged arrows mark onset of visible root injury at  $23^\circ \text{C}$ , naked arrows at  $28^\circ \text{C}$ .

Only the graphs for the lowest concentration of acetic acid and of propionic acid and the ones for the lowest and intermediate concentrations of formic acid show any considerable conductance increase within the 8-day period, but of course the conductance of the other organic acid solutions might have increased eventually had the period been longer. To make possible satisfactory comparisons among all six acids, with reference to con-

ductance increase, it would apparently have been necessary to employ experimental periods longer than 8 days.

Turning to the changes in hydrogen-ion concentration of the organic acid solutions, these were very nearly like the corresponding changes that occurred in the mineral acid solutions, except that the original solutions were generally less acid and the amount of change was correspondingly less. The initial pH values of the three concentrations of each of the three organic acids were as follows:  $\text{HCOOH}$ , 4.5, 3.9, 3.6;  $\text{CH}_3\text{COOH}$ , 4.8, 4.5, 4.1; and  $\text{C}_2\text{H}_5\text{COOH}$ , 4.9, 4.5, 4.2. In general there was rapid decrease in acidity during the first day, the following pH values being reached:  $\text{HCOOH}$ , 5.6, 5.7, 5.8;  $\text{CH}_3\text{COOH}$ , 5.5, 5.3, 5.3; and  $\text{C}_2\text{H}_5\text{COOH}$ , 5.3, 4.5, 4.3. Subsequently the decrease in acidity continued, but was more gradual, the solutions attaining a value of about 6.0 by the end of 3 days (intermediate and highest concentration of  $\text{HCOOH}$  and all three concentrations of  $\text{CH}_3\text{COOH}$ ) or at the end of 4 days (lowest concentration of  $\text{HCOOH}$  and all concentrations of  $\text{C}_2\text{H}_5\text{COOH}$ ), after which pH was approximately maintained. These times are indicated by the positions of the circles on the graphs of figure 5.

#### PHYSIOLOGICAL EFFECTS

**CONTROLS.**—On the roots of the control plants, in nutrient solution, lateral branches always appeared within the first day of the experiment and these subsequently increased in number and enlarged as the primary root continued to elongate. On the second day, when the hypocotyl was well extended, the cotyledons diverged, and later the plumule opened and elongation of the epicotyl began. No chemical injury was apparent and these plants appeared to be quite healthy throughout the entire experimental period of 14 days, excepting that, being in darkness or very weak light, they were markedly etiolated.

**PLANTS WITH ROOTS IN WATER.**—Seedlings with roots in  $\text{H}_2\text{O}$  developed more slowly than those with roots in nutrient solution. Development of lateral roots was similar to that in nutrient solution except that these did not appear until after 2 days and they did not elongate so much. After 2 days also the cotyledons began to diverge and gradually development of the epicotyl proceeded. Injury at the tips of the roots was evident after about 9 days at the lower temperature and after about 4 days at the higher temperature, as is indicated by the arrows of figure 1. Injury to hypocotyls followed in many cases.

**PLANTS WITH ROOTS IN MINERAL ACID SOLUTIONS.**—In solutions of mineral acids development was still more retarded than in water and injury was apparent earlier. Injury was first noticeable as a slight constriction in the primary root, 1 to 2 mm. from the tip. The tip region soon became

flaccid and translucent, then very soft and brown. These changes gradually spread upward until, in the most severe cases, only the upper centimeter or less of the root was apparently free from necrosis. Injury to the hypocotyl usually followed soon after the onset of root injury. It was first evident as a translucent region 1 to 2 cm. long, about the middle of the hypocotyl, and necrosis spread both upward and downward. Subsequently the injured region of the hypocotyl turned brown and collapsed, sometimes with exudation, whereupon the cotyledons and plumules drooped. The plumule developed only slightly or not at all.

Development in the mineral acid solutions was roughly in inverse relation to the original concentration of the solution. Neither primary root nor hypocotyl elongated to any considerable extent in the solutions of highest concentration and both showed less elongation in the solutions of intermediate concentration than in the most dilute solutions. Lateral roots appeared after 2 days in all cases; in solutions of lowest concentration they developed throughout practically the entire length of the primary root, in solutions of intermediate concentration they were confined to the upper two-thirds of the main root, and in solutions of highest concentration they were confined to the upper one-third or less. When the region of their development was restricted they were unusually crowded. At the end of the experimental period they were longer in all mineral acid concentrations than in water, and their length was greater as the region supporting them was shorter. At either temperature, injury to the roots became evident earlier and progressed more rapidly as solution concentration was higher, and for any tested concentration it appeared earlier and progressed more rapidly at the higher than at the lower temperature. At the end of the experimental period injury in the least concentrated solutions was confined to the terminal region of the primary root, in solutions of intermediate concentration it occupied from one-third to one-half of the length of the main root, and in solutions of highest concentration the primary root was visibly injured throughout practically its entire length. Injury to the hypocotyls also appeared earlier and progressed more rapidly as the solution was more concentrated and as the temperature was higher. If the times required for the onset of root injury in these solutions (as shown by the positions of the arrows in figures 2 to 4) are considered roughly as reciprocally proportional to the toxicity of the solutions, then it appears that, for the two lower concentrations at the lower temperature, the decreasing order of toxicity for these three mineral acids was  $\text{HNO}_3$ ,  $\text{H}_2\text{SO}_4$ ,  $\text{HCl}$ . For the highest concentration at this temperature  $\text{HNO}_3$  and  $\text{H}_2\text{SO}_4$  were about alike and produced injury sooner than  $\text{HCl}$ . For the lowest and for the intermediate concentration at the higher temperature  $\text{HNO}_3$  and  $\text{HCl}$  were about alike and produced injury more promptly than  $\text{H}_2\text{SO}_4$ . Finally, all three acids were approximately alike, on the basis of this time criterion, for the

highest concentration at the higher temperature since all had produced injury within the period before the first examination, but in these cases injury was noticeably more severe in  $\text{HNO}_3$  solutions than in the other acids. It thus appears that both temperature and concentration took part—along with the chemical characteristics of the acids—in determining the promptness of the onset of injury.

**PLANTS IN ORGANIC ACID SOLUTIONS.**—Development was more rapid and more extensive in the solutions of organic acids than in the corresponding solutions of mineral acids. With formic and acetic acids development was somewhat greater in the lowest concentrations than in the others; with propionic, however, development in the solution of intermediate concentration equalled or exceeded that in the most dilute solution, and development in the most concentrated solution was less rapid than in either of the other solutions. By the second day lateral roots had emerged in all cases. They appeared earlier, however (after only 1 day), in the most concentrated solutions of formic acid and acetic acid at the higher temperature, and in all solutions of propionic acid at this temperature. Like the mineral acids tested, the organic acids permitted the development of laterals throughout most of the length of the primary root in solutions of lowest concentration. As the solution was more concentrated laterals were increasingly restricted to the older region of the primary root, but the region bearing laterals was not so limited as under the corresponding influence of the mineral acids. As with the inorganic acid solutions, but to a lesser extent, lateral roots were finally more closely crowded and longer the more restricted was the region from which they arose.

Injury by the organic acids was similar, in inception and nature, to that produced by the mineral acids, but for any tested concentration and temperature it generally appeared somewhat later with the most rapidly injurious organic acid than with the least rapidly injurious mineral acid, as is shown by comparing the positions of the arrows on the graphs of figures 2 to 5. (It should be noted that in figure 5 winged arrows refer to the lower temperature while naked arrows refer to the higher temperature.) Acetic acid generally appears to have been less promptly injurious than either of the other organic acids, which were apparently about alike in this respect. In general, injury by any solution was less prompt at the lower than at the higher temperature and it appeared sooner as the concentration was higher.

#### THE SIX ACIDS AND $\text{H}_2\text{O}$ COMPARED AS TO TOXICITY

Although the available data are inadequate for a satisfactory comparative study of the degrees of toxicity of all six acids, it may be of interest to summarize the lengths of time required for the onset of visible root injury, as follows:

1. At 22° or 23° C. the descending order of indicated toxicity for each concentration is:
  - a. For lowest concentration:  $\text{HNO}_3$  (3.5 days),  $\text{HCOOH}$  and  $\text{CH}_3\text{COOH}$  (5 days),  $\text{H}_2\text{SO}_4$  (6 days),  $\text{HCl}$  (8 days).
  - b. For intermediate concentration:  $\text{HNO}_3$  (1 day),  $\text{HCOOH}$  (3 days),  $\text{H}_2\text{SO}_4$  (4 days),  $\text{CH}_3\text{COOH}$  (5 days),  $\text{HCl}$  (6.5 days).
  - c. For highest concentration:  $\text{HNO}_3$  and  $\text{H}_2\text{SO}_4$  (1 day),  $\text{HCOOH}$  (2 days),  $\text{HCl}$  (2.5 days),  $\text{CH}_3\text{COOH}$  (3 days).
2. At 28° C. the descending order of indicated toxicity for each concentration is:
  - d. For lowest concentration:  $\text{HCl}$  (2 days),  $\text{HNO}_3$  (2.5 days),  $\text{HCOOH}$  and  $\text{C}_2\text{H}_5\text{COOH}$  (3 days),  $\text{H}_2\text{SO}_4$  (4 days),  $\text{CH}_3\text{COOH}$  (5 days).
  - e. For intermediate concentration:  $\text{HNO}_3$  and  $\text{HCl}$  (1 day),  $\text{H}_2\text{SO}_4$  and  $\text{C}_2\text{H}_5\text{COOH}$  (2.5 days),  $\text{HCOOH}$  (3 days),  $\text{CH}_3\text{COOH}$  (4 days).
  - f. For highest concentration:  $\text{HNO}_3$ ,  $\text{H}_2\text{SO}_4$ ,  $\text{HCl}$  and  $\text{HCOOH}$  (1 day),  $\text{C}_2\text{H}_5\text{COOH}$  (1.5 days),  $\text{CH}_3\text{COOH}$  (2 days).

For each combination of temperature and concentration the reciprocal of the injury-time for each acid may perhaps be taken as an index of its relative toxicity. If for each combination all six reciprocals are expressed in terms of the reciprocal for  $\text{HNO}_3$  and if the resulting relative toxicity values for each acid are averaged, the final outcome is a single series of tentative toxicity indices that may represent all the tested combinations of temperature and concentration; that new series is as follows:  $\text{HNO}_3$ , 1.00;  $\text{HCl}$ , 0.71;  $\text{H}_2\text{SO}_4$ , 0.66;  $\text{C}_2\text{H}_5\text{COOH}$ , 0.63;  $\text{HCOOH}$ , 0.61;  $\text{CH}_3\text{COOH}$ , 0.41. The corresponding value for  $\text{H}_2\text{O}$  is 0.22. According to these averages, it appears on the whole: (a) that  $\text{HNO}_3$  was the most toxic, while (b)  $\text{CH}_3\text{COOH}$  was least toxic of the six acids considered, (c) the former being about 2.5 times as toxic as the latter; (d) that  $\text{HCl}$  and  $\text{H}_2\text{SO}_4$  were nearly alike in toxicity (about 69 per cent. as toxic as  $\text{HNO}_3$ ); (e) that  $\text{C}_2\text{H}_5\text{COOH}$  and  $\text{HCOOH}$  were nearly alike in toxicity (about 62 per cent. as toxic as  $\text{HNO}_3$ ), (f) only slightly less toxic than  $\text{HCl}$  and  $\text{H}_2\text{SO}_4$ , and (g) about 50 per cent. more toxic than  $\text{CH}_3\text{COOH}$ ; and, finally, (h) that  $\text{H}_2\text{O}$  appeared to be about half as toxic as  $\text{CH}_3\text{COOH}$  and about one-fifth as toxic as  $\text{HNO}_3$ . While these statements seem to agree in general with some conclusions that are suggested by superficial scrutiny of the graphs and their appended arrows, it must be remembered that they are intended only to represent a sort of summary of the toxicity phase of the present study, for it is almost certain that the degrees of toxicity of these acids in any test depend on temperature and concentration as well as on chemical nature.

### Conclusion

The initial reduction in conductance of these acid solutions was evidently due to absorption of ions by the roots. This absorption was probably accompanied by some exosmosis in all cases, as was shown for  $\text{H}_2\text{O}$ .



While it is conceivable that the conductance of a solution might be diminished by exosmosis alone, if the materials extruded from the roots were of such nature as to remove from solution the ions present, consideration of the nature of the solutions examined in this study and of the kinds of material that might possibly escape from plant roots leads to the conclusion that the observed conductance decrease must have been due to direct removal of ions by the plants. This was no doubt accomplished principally by absorption of material into the cells, but adsorption of ions on to root surfaces may have occurred also. Since solutions of all the acids tested were reduced in conductance, and hence in ion concentration, it seems safe to conclude that ions were absorbed from both inorganic and organic acid solutions.

The amount of net absorption from the several solutions seems to have varied directly with ion supply. For any acid, the higher the ionic concentration of the original solution, the greater and more rapid was the removal of electrolytes. And among the six acids tested, the greater the degree of ionization, in general, the more rapid was net absorption. A general index of absorption from these acid solutions under all the conditions represented by these experiments may be derived by expressing the average amount of net absorption from each concentration of each acid in terms of the average net absorption from the corresponding concentration of  $\text{HNO}_3$ , and averaging the three values for each acid. This index is as follows:  $\text{HNO}_3$ , 1.0;  $\text{H}_2\text{SO}_4$ , 0.83;  $\text{HCl}$ , 0.77;  $\text{HCOOH}$ , 0.73;  $\text{CH}_3\text{COOH}$ , 0.30; and  $\text{C}_2\text{H}_5\text{COOH}$ , 0.12. That is, considering the average of the results of the several experiments, there was greatest absorption from  $\text{HNO}_3$  solutions, and least from those of  $\text{C}_2\text{H}_5\text{COOH}$ . Net absorption from the organic acid solutions was generally less than from the corresponding concentrations of mineral acids, but  $\text{HCOOH}$  was only slightly below  $\text{HCl}$  and  $\text{H}_2\text{SO}_4$  in this respect. This is in general agreement with the order of dissociation of these acids.

Absorption was, however, apparently influenced by other factors to some extent. Examining the results for the two temperatures separately it may be seen that, for the several mineral acids at the lower temperature, net absorption from  $\text{HNO}_3$  solutions was greatest and that from  $\text{H}_2\text{SO}_4$  solutions was least, net absorption from  $\text{HCl}$  being intermediate. This order of penetration ( $\text{HNO}_3 > \text{HCl} > \text{H}_2\text{SO}_4$ ) is in agreement with DAVIDSON and WHERRY's (9) results with wheat seedlings. On the other hand, in the present study the order of penetration at the higher temperature is:  $\text{HNO}_3 > \text{H}_2\text{SO}_4 > \text{HCl}$ ; this is the order reported by HIND (15) from her experiments with potato tuber. Net absorption was apparently increased by rise in temperature in the case of all solutions of  $\text{H}_2\text{SO}_4$  and the intermediate concentration of  $\text{HNO}_3$ , but in the case of the other solutions (namely, all concentrations of  $\text{HCl}$  and the lowest and highest con-

centrations of  $\text{HNO}_3$ ) net absorption was apparently decreased. DELF (10) and SKEEN (20) found the permeability of plant tissues to increase with rise in temperature, and STILES and JØRGENSEN (24) report greatly augmented absorption of hydrogen ion under similar conditions. ECKERSON (13), on the other hand, reports that the effect of rise in temperature on permeability of root cells varied according to the species considered. Studies on this subject reported in the literature have been carried out with a variety of materials and by means of a variety of experimental procedures. It is safe to presume that rates of penetration of materials into plant roots, and rates of absorption, depend on the kind and condition of the roots as well as on temperature and the nature of the solutions from which the absorbed substances are derived.

The pronounced decrease in hydrogen-ion concentration which accompanied the initial decrease in conductance of these solutions may be taken as evidence that there was rapid absorption of hydrogen ion in the early part of each experimental period. As was the case with absorption in general, this was always greater the higher the concentration of the original solution, and greater the first day than in any succeeding day. Absorption of hydrogen ion by plant cells has been reported by many observers (5, 14, 18, 19, 23).

The toxicity of the hydrogen ion has long been recognized. About forty years ago KAHLENBERG and TRUE (17) pointed out that the toxic influence exerted upon plants by dilute solutions of strong acids was probably largely due to this ion. Since that time BREAZEALE and LeCLERC (3), HOAGLAND (16), and DUNN (12), among others, have reported on acid toxicity for plant tissues. As to the effect of such toxicity within plant cells, ADDOMS (1) observed that the protoplasm of root hairs injured by an acid medium appeared to have been coagulated.

It seems probable that the toxic effects produced by the acid solutions of the present study were due chiefly to the hydrogen ion. Similar injuries appeared in all experimental plants and this was the only ion, other than the hydroxyl ion, which was common to all solutions. Also the hydrogen ion is more active, and probably more toxic, than any of the anions present.

In general, degree of toxicity followed hydrogen-ion concentration. For any acid the higher the concentration and the greater the ionic absorption therefrom, the more promptly did injury occur, and in the acids as a group it was generally true that the more completely dissociated the acid the more toxic it was. Propionic acid was, however, more toxic than would be expected from its ionic concentration or from the amount of ionic absorption that occurred from its solutions. Apparently the propionic molecule also was toxic. This is in agreement with TRUE's (25) idea that the harmful action of formic and acetic acids is due principally to hydrogen ion, whereas that of propionic acid is due chiefly to the undissociated molecule. Other

workers also have attributed the toxicity of organic acid solutions to undissociated molecules (6, 8, 12). Even in the case of the acids whose toxicity seems to have been fundamentally due to excess of hydrogen ion, other ions or molecules may not have been without influence on toxicity.

The toxicity of these acids was generally greater at the higher temperature than at the lower. Injury was apparent earlier and the plants became more severely affected at the higher temperature. SKEEN (21) also has found toxicity to be increased by increase in temperature in some cases.

Although, as has been intimated, it appears likely that the initial decrease in conductance of the acid solutions was fundamentally due to hydrogen-ion absorption by the roots, there is indirect evidence that other ions and molecules also were absorbed. Furthermore there seems to be no reason to doubt that there was in all cases some exosmosis of ions or molecules, or both. That the initial lowering of solution conductance ceased—generally after 1 to 3 days for the mineral acids, and after a somewhat shorter time for the organic acids—must have been due to decreased absorption, to increased exosmosis, or to both together. Either of these alterations in the rate of movement of material across the root periphery may well have been occasioned by increasing toxic action. Indeed this increased exosmosis has been used as a criterion of injury by some workers (8, 22). With the mineral acids excessive exosmosis from the poisoned plants eventually became greatly pronounced, as was shown by increase in solution conductance, but no rapid outburst of electrolytes had occurred at the time the experiments with the organic acids were discontinued. Nor did rapid exosmosis occur within 8 days in solutions of aromatic acids tested in a similar way by MOTHER CHRYSOSTOM (8).

In those cases where conductance increased rapidly toward the end of the experimental period, the sudden increase in exosmosis that is indicated may have been due to a corresponding sudden increase in outward permeability of the root cells to electrolytes, such as might result from some sorts of poisoning, or perhaps poisoning may first have caused general protoplasmic coagulation, followed at length by a sudden disintegration. It is possible that the anions or undissociated molecules of the organic acids acted in some way to retard the poisoning process or hinder protoplasmic disintegration.

### Summary

1. Roots of *Lupinus albus* were found to absorb electrolytes from very dilute solutions of certain inorganic acids— $\text{HCl}$ ,  $\text{H}_2\text{SO}_4$ ,  $\text{HNO}_3$ —and certain organic acids— $\text{HCOOH}$ ,  $\text{CH}_3\text{COOH}$ ,  $\text{C}_2\text{H}_5\text{COOH}$ . Absorption was greater in amount and more rapid the higher the original concentration of the solution. For any tested concentration it was greater as dissociation was more nearly complete.

2. There was apparently a rapid absorption of hydrogen ion, probably accompanied by some absorption of other ions, of molecules, or of both.

3. Injury to the plants resulted in all cases. This is probably to be attributed to absorbed hydrogen ion in each instance except that of propionic acid, whose undissociated molecules seem to have been largely responsible for the injury.

4. In many cases rapid exosmosis of materials from the roots eventually occurred, indicating increased permeability of cell membranes or rapid disintegration of cell contents.

5. Influence of temperature, solution concentration, and hydrogen-ion concentration on absorption and exosmosis and on growth and development of the seedlings are among the topics discussed.

This problem was suggested by Professor RODNEY H. TRUE, Director of the Morris Arboretum, who maintained a continuous interest throughout the study. In the analysis of the results and in the preparation of this paper the writer received many helpful suggestions from Dr. BURTON E. LIVINGSTON, of the Johns Hopkins University, and from Dr. CHARLES A. SHULL, of the University of Chicago.

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# UREASE DISTRIBUTION IN PLANTS: GENERAL METHODS<sup>1</sup>

S A M G R A N I C K <sup>2</sup>

## Introduction

Although numerous studies have been made on enzymes with the purpose of elucidating the intermediary metabolism of various substances, such as the fats, carbohydrates, and proteins, the metabolic changes undergone by the enzymes, that is, their synthesis, storage, and decomposition have received scant attention. Of course the metabolism of an enzyme could be discussed only superficially until a few years ago, because its composition was totally unknown. The discovery by SUMNER (22) that an enzyme, urease, possessing the properties of a globulin, could be obtained in a crystalline state, marked the beginning of a new era in enzyme chemistry. Likewise, the methods developed by LINDERSTRØM-LANG and HOLTER (13) for the application of quantitative histochemistry to enzymes, have led away from a study of gross structures to a more intimate study of tissues and even of cells.

The purpose of the present investigation was to follow the changes in the content and distribution of urease throughout the life cycles of the two leguminous plants which, so far as known, are the richest in their content of this enzyme, namely, the soy bean and the jack bean. The most sensitive tests for most proteins are valueless below a dilution of one part in 10,000. Urease can be detected in much greater dilutions than this. Advantage was taken of the catalytic nature of the enzyme to select certain histological staining reagents which would reveal the presence of the enzyme directly within the cells. By means of these reagents it is possible to study the changes of urease activity taking place during the histological development of the plant. Since the number of cells per organ (for example, the leaf) does not change greatly after an early stage in the development of that organ, one can study, by analyzing organs of different ages, the progressive changes in urease content of cells of increasing age.

It was hoped that the data would not only reveal the course of urease metabolism but would also clarify a number of other problems as well. Considering urease as a protein, one can follow the metabolism of a protein of specific constitution throughout the life history of a plant. If the assumption be made that the main portion of the protoplasmic framework of a cell

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This is the first of a series of three papers on urease distribution in *Canavalia ensiformis* and *Soja max*. More extensive data and discussion of this subject may be found in the writer's thesis in the University of Michigan library.

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consists of proteins, it might be possible, by following the changes in urease activity of the cells under various environmental conditions, to get some information about the changes occurring in the protoplasmic framework. Also, since urease has been found in plants of every division of the plant kingdom (4, 27), it becomes interesting to determine what fundamental relation the enzyme has to the nitrogenous metabolism of the plant.

The reaction catalyzed by urease, as shown by MACK and VILLARS (15) and by later investigators, is the hydrolysis of urea to ammonium carbamate. Urea itself is very slightly ionized. Ammonium carbamate is alkaline; it is unstable, especially in acid solutions, and undergoes spontaneous hydrolysis to ammonium carbonate. Two general methods have been used in this investigation: a histological method which depends on the increase in alkalinity as urea is hydrolyzed by urease; and a quantitative method based on the determination of the ammonia produced.

### Histological urease determinations

An attempt was made to find a method which would give direct visual evidence of the exact location of urease in the tissues. WAGENAAR (25), SEN (20), and WASICKY and KRACH (27) used a number of reagents. The last-named investigators found that reagents for the determination of  $\text{NH}_4^+$  such as Nessler's reagent, chloroplatinic acid, etc., were much less sensitive than pH indicators, and suggested the use of an alcoholic haematoxylin solution as a suitable indicator. After testing a number of methods, the writer came to the same conclusion as WASICKY and KRACH, namely, that the change of hydrogen-ion concentration was the most sensitive method upon which to base the detection of urease.

Two histological methods were used, the indicator and the lake methods, both of which depend upon the detection of urease by an increase in alkalinity.

### INDICATOR METHOD

A number of factors which would influence the sensitivity and accuracy of the indicator method had to be considered. These may best be appreciated by imagining an enzyme point in a cell at which urea molecules are undergoing decomposition. The first molecules of ammonium hydroxide released at this point will react with various buffers in the immediate vicinity of the enzyme point. This ammonium hydroxide will therefore not show up as a change in hydrogen-ion concentration and the indicator will not be affected. After sufficient ammonia has been produced the pH of that region will shift toward the alkaline side. The rapidity of the pH change will then partially depend on buffer capacity. It is readily seen that the most sensitive indicator to choose will be one whose midpoint (pK) will be that of the

hydrogen-ion concentration of the cells. It is therefore necessary to know the hydrogen-ion concentration of the tissues examined. The series of indicators recommended by SMALL (21) was found suitable for an approximate determination of the pH of the tissues.

**INDICATORS USED.**—Because of their visually favorable change in color from yellow at the acid end to dark blue at the alkaline end, the indicators brom-cresol purple, brom-thymol blue, brom-cresol green, etc., were used in a 0.2 per cent. aqueous solution to detect enzymic action. They were adjusted to their midpoint with 0.05N acid or alkali. Although these indicators showed the presence of the slightest traces of enzyme their colors were not intense, but quite diffuse, so that only a vague notion could be obtained as to the enzyme location. Haematoxylin, on the other hand, was found to be excellent for the purpose. Saturated aqueous solutions of haematoxylin were prepared fresh every three or four days since changes take place in this indicator on standing. It is yellow at the acid end and intensely red at the alkaline end of its range, with a midpoint around pH 6.5. Besides showing a marked color change with change in pH, the dye in its alkaline form appears to be quite localized in a tissue. It was found upon further study that the permeability of the cells is much less to alkaline haematoxylin than to the neutral or acid haematoxylin. The yellow-colored (acid) haematoxylin could be washed out readily with water, without as readily removing the red-colored haematoxylin.

**PERMEABILITY OF CELLS TO INDICATORS AND UREA.**—If one is to judge the location of the enzyme by a change in color of an indicator within the cells, one must know that the indicator and urea solutions have penetrated all the different cells of a tissue section. The penetration of the indicator is quite readily determined by noting its distribution. Generally, the dye colors all of the cells of a tissue section. However, in certain instances, as, for example, in sections of the collet region of jack bean seedlings, some pith cells appear to be less readily permeable to the dye than their neighbors. In such instances, the dye solution is allowed to remain on the section for a few minutes longer until these cells also are colored.

It has generally been considered that cells are readily permeable to urea. HÖFLER and STIEGLER (8), HÖFLER and WEBER (9), and WEBER (28) have shown, however, that the penetration of urea into the cells of various tissues of the same plant and even of the same tissue was different; permeability toward urea was also found to differ with the age and the activity of the cells. By treating cells of various tissues with 20 per cent. ethyl alcohol in order to destroy the plasma membranes and permit unhindered diffusion of urea into all of the cells, it was found that the controls and alcohol-treated sections showed no marked differences in permeability toward urea. In a few instances the alcohol-treated sections appeared to contain slightly less



enzyme, but the distribution even in these sections was the same as in the controls. Perhaps even the least permeable cells allowed sufficient urea to enter from the aqueous urea solution. SMALL (21) reports an increased permeability and outward diffusion of salts from cells treated with 20 per cent. alcohol. The enzyme apparently is not rapidly destroyed in 20 per cent. alcohol. Indeed, sections of jack bean cotyledons immersed for a few minutes in a 95 per cent. alcoholic solution still possessed active urease.

**BUFFER CAPACITY OF CELLS AND REAGENTS.**—As already indicated, it is necessary to take into account the buffer capacity of the cells studied because, if ammonia is released at the enzyme points, the buffers of the cell will neutralize the ammonia; the rate of indicator change toward the alkaline side will therefore depend upon the buffer capacity of the cell. Although one cell may have a greater enzyme content than another, yet the amount of enzyme in the first cell may be masked by its greater buffer capacity. Likewise, it is possible that a cell may possess both the enzyme and a high buffer capacity, whereas a neighboring cell may contain no enzyme but have a low buffer capacity; in this instance ammonia would diffuse to the neighboring cell containing no enzyme and change the indicator to the alkaline side and apparently indicate the presence of the enzyme. It was found that the meristematic tissues, including the cambium, are most highly buffered, next come the smaller parenchyma cells, then the larger parenchyma cells of the cortex and pith, and finally the xylem cells.

At first glance it would seem that, because of the impossibility of controlling the tissue buffers, the indicator method would be useless for the exact localization of the enzyme in specific cells and tissues. Fortunately, this is not generally so. In sections of the apical bud, where buffer capacity must be considered, it is found that the apical primordium and the axillary primordia, which are the most highly buffered tissues, are also the tissues containing the most enzyme. On the other hand, the buffer capacity in the cambium does not permit a comparison of the enzyme content of that tissue with the content of neighboring parenchyma cells. This is true in the stem below the third node (first node above the cotyledonary node) of the seedlings. In these parts, the enzyme content of the pith and cortex is relatively high, but none can be detected with certainty in the cambium or its derivatives. Above the third node, however, the pith and cortical parenchyma contain no enzyme detectable by the indicator method, and the cambium also gives a negative reaction. That the cambium and its derivatives contain no urease is a generalization that may be made, while keeping the limitations of the indicator method strictly in mind.

Since the enzyme is detected by a change in the hydrogen-ion concentration, it is essential that the reagents used should also have little or no buffering power. Determinations of the buffer capacity of the 0.2 per cent.

dye solutions and the 1 per cent. urea solution showed it to be very slight in these solutions. As contrasted with the buffer capacity of any of the parenchyma cells examined, that of the indicators and of the urea solutions is negligible.

**TIME FACTOR.**—It is evident that if urease is permitted to act for a longer time more ammonia will be formed and the tissue will become more alkaline. The maximum time for observation is limited by the following considerations: In the first place, carbonic acid is constantly being produced, and neutralizing a portion of the ammonia released. In certain tissues, the enzyme content is so slight that more  $\text{CO}_2$  than ammonia is produced and the acidity of the tissue increases, as has been determined in a few experiments with a sensitive glass electrode apparatus. In the second place, the enzyme appears to be slowly inactivated. Thirty minutes was found to be the maximum time that could be used for these histological determinations.

**TISSUE SECTIONS.**—For approximate estimates of the enzyme, sections were made with a hand razor. If delicate structures were to be examined or accurate comparisons of sections were to be made, a freezing microtome was used at first. It was soon found, however, that small pieces could be imbedded in paraffin of low melting-point and rapidly cooled, without appreciable destruction of the enzyme. The time, from removing the material from the plant until the section was ready for study under the microscope, varied between 15 and 20 minutes.

#### LAKE METHOD

Although a particular indicator will permit the detection of urease by the first appearance of color change, the ammonia generated at the enzyme points diffuses so rapidly into the surrounding cells that the color of the alkaline indicator becomes quite diffuse. The ammonia may also invade tissues containing no enzyme and the indicator test may then give spurious results. It is therefore necessary, for sharp localization of the enzyme, to secure a reagent which will form a non-diffusible precipitate at the enzyme points. At the same time it is desirable to poison the enzyme points in order to prevent ammonia from being continuously liberated and invading the neighboring tissues. It is necessary, moreover, that the precipitate be readily visible within the cells. Of the large number of reactions tested, the formation of a lake (an adsorption complex of a dye with the hydroxide of a heavy metal) satisfies these three requirements.

The inactivation of a urea solution with various metals has been studied by SCHMIDT (19), and more recently by JACOBY (11). The following was found to be the order of poisoning effectiveness:  $\text{Cu}^{++} > \text{Hg}^{++} > \text{Ag}^+ > \text{Ni}^{++} > \text{Fe}^{++} > \text{Zn}^{++} > \text{Pb}^{++}$ . The last elements had only comparatively slight effect on urease activity.

The formation of metallic hydroxides at various hydrogen-ion concentrations has been studied by BARTON (3) and others. They find that the hydrogen-ion concentrations at which the hydroxide will form is a characteristic of that metal. For example,  $\text{Ni}^{++}$  will form its hydroxide when added to a solution whose hydrogen-ion concentration is pH 6.7 or above. The hydroxides of the heavy metals are generally very insoluble gelatinous precipitates which would be difficult to discern when formed within the cells. With the proper dye adsorbed, it is possible to detect traces of metallic hydroxides.

The application of the principle outlined is best illustrated by a specific example. Microtome sections of an apical meristem are treated with aqueous haematoxylin. This is allowed to remain on the sections for a few minutes until it has penetrated all of the cells. Excess fluid is drained off and a drop of a 1 per cent. urea solution and a drop of 0.05 M  $\text{NiCl}_2$  are now added. A cover slip is placed over the sections, excess fluid is drained off, and the slide is examined immediately with a microscope to note the distribution of the deep blue color of the nickel hydroxide-haematoxylin lake.

The order of adding the reagents depends on the amount of enzyme present. If the enzyme is highly concentrated, as it is in the mature cotyledons, it is advisable to use a solution of nickel chloride and in addition a trace of cupric or mercuric chloride to poison the enzyme more rapidly. This solution is added immediately after the urea. If, on the other hand, the enzyme content is very low, it may be desirable to add a very dilute nickel chloride solution some time after the urea solution is added. The enzyme action will convert the yellow haematoxylin to a red-purple color. On addition of the dilute nickel chloride solution, this color will become deep blue and quite sharply delimited from the yellow of the surrounding tissues.

### Quantitative urease determinations

An excellent discussion of various quantitative methods for the determination of urease is given by EULER (4). After several methods had been tested, a colorimetric method was chosen. The ammonia produced by a piece of tissue containing urease, after the latter has acted on a urea solution for a certain time, is driven over with a rapid current of air into an acid solution in which the ammonia is determined. With some modifications the method is essentially that described by VAN SLYKE and CULLEN (29).

A weighed portion of ~~from~~ tissue is finely ground in a porcelain mortar with 1 to 2 cc. of M phosphate buffer (pH 7.2), with quartz sand when necessary. The mixture is transferred to a volumetric flask of appropriate capacity, made up to volume with distilled water at 30° C., and the flask placed in the water bath (30° C.). Into each of the several reaction tubes

are pipetted 1 cc. of phosphate buffer, 1 cc. of freshly prepared 2 M urea solution, and the requisite amount of distilled water. The amount of distilled water is so chosen that the final volume of solution in the reaction tube is exactly 10 cc. The tubes are also placed in the water bath. After they have come to the temperature of the bath, an aliquot of tissue solution is pipetted into each tube. The time from the beginning of grinding until the tissue is added to the reaction tube varies between 10 and 15 minutes. Exactly 30 minutes (stop watch) after the tissue is placed in the tube the urease is inactivated by the addition of 0.5 cc. of 2.5 N  $\text{H}_2\text{SO}_4$ . The acid solution serves a twofold purpose. It not only inactivates the enzyme solution completely, but also decomposes the ammonium carbamate which was produced by the hydrolysis of urea. (Alkali does not inactivate urease completely.) After 5 minutes, the reaction tube is placed in the aeration apparatus. A few drops of caprylic alcohol and 5 gm. of anhydrous  $\text{K}_2\text{CO}_3$  are rapidly added, the tubes stoppered, and suction from the water pump is applied. Control tubes containing tissue but no urea are treated in all respects like those described above. After all the ammonia has been driven over into the tube containing acid, it is determined colorimetrically with a Duboscq colorimeter. If the ammonia content is above 0.05 mg., the customary Nessler's reagent is used. For determinations of less than 0.05 mg.  $\text{NH}_3$ , the sodium phenate reagent as modified by VAN SLYKE and HILLER (30) was used.

The sum of the ammonia present in the tissues and in the reagents, subtracted from the total ammonia values obtained, gives the ammonia produced through the decomposition of urea by the urease of the tissues.

Using a maximum of 0.5 gm. of tissue and a time-period of 30 minutes for reaction the method permitted the determination of a wide range of enzyme activity. In actual practice, the enzyme values ranged from 30 U.U. down to 0.0001 U.U. where *U.U.*, the urease unit, is a quantity of urease which will produce one milligram of  $\text{NH}_3$  per minute under the experimental conditions employed.

#### FACTORS CONCERNED IN QUANTITATIVE UREASE DETERMINATIONS

The quantity of urease is determined indirectly by measuring the rate of formation of ammonia from urea under certain standardized conditions. These conditions were chosen and various other factors examined in relation to their influence on urease activity. Although numerous publications have dealt with the quantitative measurements of the activity of a particular tissue containing an enzyme few bear upon the difficulties that arise in an attempt to make comparisons of the quantity of enzyme in the same tissue at different stages of development, or in different tissues. A number of factors cannot be adequately controlled. However, an approximation to the true values of enzyme activity can be obtained.

A number of methods have been used by investigators to prepare the plant material for enzyme analysis. For determinations of the total enzyme content it is obviously useless to extract the enzyme, since extraction is never complete; it is different for different materials; and during extraction inactivation of the enzyme occurs.

**EFFECT OF DRYING.**—It is important to learn whether tissues may be dried without causing changes in enzyme activity. PETT (17) has studied the effect of drying germinated wheat seeds and concludes that the dipeptidase activity increases on drying. Since he used a glycerol extract of the material for his enzyme determinations, it may be that drying merely increased the amount of enzyme extracted by the glycerol, and not the dipeptidase activity.

In the following experiments the material was spread out on filter paper and allowed to dry at room temperature (approximately 22° C.). The urease content was determined immediately and after several days of drying.

TABLE I  
EFFECT OF DRYING ON UREASE ACTIVITY

PLANT MATERIAL	TIME AFTER COLLECTING	MOISTURE	U.U. PER BEAN
		%	
Soy beans, nearly mature (beans removed from pods; 10 used for each determination)	Immediate	61.0	1.01
	1 day	35.2	0.988
	7 days	10.1	0.976

TABLE II  
EFFECT OF DRYING ON UREASE ACTIVITY

PLANT MATERIAL	TIME AFTER COLLECTING	U.U. PER GM. OF ORIGINAL FRESH WEIGHT
Jack bean leaflets (young; 5-8 cm. long; 15 used for each determination)	Immediate	0.0542
	6 days	0.0344

The data in table I show only a slight decrease in urease content during slow drying of nearly mature soy beans. In the young leaflets of jack bean (table II), however, air-drying decreased the urease activity 37 per cent. Because of these results it was decided that all analyses should be made on fresh tissues.

**EFFECT OF MACERATING.**—To determine what effect crushing and macerating the tissues would have on urease activity, opposite leaflets (with petioles removed) of jack bean plants were used. These leaflets possess numerous small stomata on the under surface but none on their upper surface. Whole leaflets, leaflets cut into 5-mm. squares, and macerated leaflets were compared

as to their urease activity. It was found that with a 0.2 M urea solution the whole leaf showed an activity one-thirtieth that of the macerated tissue. The 5-mm. squares of leaflets had an intermediate value. Infiltration of whole leaflets with urea by means of suction, and increasing the length of time for reaction on the urea solution to 60 minutes, increased the indicated urease values of whole leaflets to one-fourth that of macerated leaflets. It appeared probable that the lower value of urease activity, if whole leaflets were used, was because of the inability of the urea solution to come into contact with all of the available enzyme points in the cells. It was thought possible that the permeability of the plasma membranes might be increased by the addition of 1 and 2 per cent. ethyl ether or ethyl chlorhydrin to the urea solution. Determinations on macerated, as well as on whole tissue, showed, however, that these chemicals, at the concentrations used, inhibited urease activity from 15 to 25 per cent. No increase in urease activity was observed with leaf-tissue mash suspended in 5 per cent. sodium chloride. Another series of experiments was performed to determine whether very high urea concentrations would not give values for urease activity of the whole leaf approximating those of macerated leaves. Using 1 to 4 M urea solutions, it was found that the activity of urease in whole leaves, especially at the highest urea concentrations, approximated that of macerated leaves. It may be concluded that maceration of the jack bean leaves causes no detectable inactivation of urease. In this connection it is interesting to note that LINDERSTRØM-LANG and HOLTER (13) report no difference in the activity of peptidase present in whole sections of barley roots as compared with crushed sections.

UREA CONCENTRATION.—An attempt was made to choose a sufficiently high urea concentration so that the amount of decomposition produced in a given time would be independent of the concentration. The data of VAN SLYKE and CULLEN (29) and of LÖVGREN (14) on soy bean extracts indicate that the rate of decomposition does not increase above a concentration of 2 M urea, although the increase above 0.2 M is slight. Results were obtained on soy bean cotyledons that were in accord with the findings of these investigators; that is, there was no appreciable increase in the decomposition rate above 0.2 M concentration of urea. A concentration of 0.2 M was then chosen for the quantitative investigations. It is a convenient concentration to handle, because a 2 M urea solution can be made up, and 1 cc. of this pipetted into a reaction tube, tissue mash and buffer added, and the whole finally diluted to 10 cc.

It was later found that the conclusion arrived at for the urea concentration of soy bean cotyledons does not hold for jack bean leaf tissue. Even above a 2.1 M substrate concentration there still is an appreciable increase in the rate of urea decomposition. With 0.1 M urea solution, 0.61 mg.  $\text{NH}_3$  was produced; with 0.7 M urea solution, 0.68 mg.  $\text{NH}_3$ ; with 2.1 M urea

solution, 0.72 mg.  $\text{NH}_3$ ; and with 3.5 M urea solution, 0.76 mg.  $\text{NH}_3$ . Obviously, one cannot speak of a maximum concentration above which all the enzyme points are being readily supplied with urea without specifying the tissue-preparation used. One may conclude that, if different tissues or plant parts, such as roots and leaves, are to be compared as to total urease content, an error of 25 per cent. may arise merely because of insufficient urea concentration; that when the same tissues or plant parts are being compared this error is eliminated.

The *temperature* chosen was 30° C. This is sufficiently high temperature to effect rapid enzymic hydrolysis, but not high enough for inactivation of the enzyme to become an important factor.

The *reaction time* was chosen as 30 minutes. This was selected after a number of preliminary trials had been made. Because the rate of inactivation of different enzyme preparations could not be controlled, the shortest time was chosen in which determinations of the lowest enzyme concentrations could be determined accurately. This of course depended on the sensitivity of the method selected for the ammonia determinations.

Some idea of the rate of urease inactivation was obtained from the following experiments. The moment that the maceration of the tissues was begun was taken as zero time. After a thorough grinding and proper dilution, the mash was placed at 30° C. and overlaid with toluene. At various intervals, an aliquot of the mash was taken for analysis, and allowed to react on the urea for 30 minutes at 30° C.

TABLE III  
RATE OF INACTIVATION OF MASH CONTAINING UREASE AT 30° C.

MATERIAL USED	TIME AFTER BEGINNING OF MACERATION		U.U. PER GM. FRESH WT.
	<i>min.</i>	<i>hr.</i>	
Soy bean cotyledons (germinated 36 hours)	{ 55		3.92
	{ 115		3.86
	{ 295		3.54
Jack bean leaflets (5-8 cm. long)	{ 49		0.0547
	{ 94		0.0550
	{ 194		0.0603
	{ 361		0.0605
		21	0.0574
		140	0.0069
Jack bean leaflets (5-8 cm. long; dried at room temperature 6 days)	{ 66		0.0344
	{ 396		0.0041

From table III it is seen that the decrease of urease activity in the soy bean cotyledons is negligible during the first few hours. It is interesting to note that the mash, made from fresh leaflets of jack bean, actually increases in urease activity during the first three hours. Here again the change of activity, during the first hour after addition of urea, is negligible. With leaflets of jack bean, dried at room temperature for six days, the rate of inactivation appears to be especially marked; no change in urease activity was observed on the addition of 0.5 cc. of saturated aqueous  $\text{H}_2\text{S}$  to the reaction tubes containing an aliquot of mash. These experiments indicate that two simultaneous processes are going on: an inactivation of the enzyme by denaturation, coagulation, or poisoning; and a dispersion, peptization, or activation of the enzyme.

The *hydrogen-ion concentration* of pH 7.2, which was produced by a phosphate buffer, was used for the urease determinations. LÖVGREN (14), using phosphate buffers, reported an optimum pH between 7.2 to 7.8 for soy bean extracts. HOWELL and SUMNER (10) found that the urease activity depended on the type of buffer and on the concentration of urea, the pH optimum increasing with decreasing urea. BACH (1) found an optimum hydrogen-ion concentration of pH 8.6 for a urease preparation extracted from an *Aspergillus*. It is quite possible that the optimum hydrogen-ion concentration for a urease preparation may depend to some extent on its concomitant impurities, as has already been found for certain enzymes. No experiments were conducted to determine whether the hydrogen-ion concentration optimum is different for different structures of the same plant. With the dipeptidases of the wheat grain PETT (17) found about the same pH-activity curve for all portions of the wheat grain that were studied.

Since ammonia is produced during urea hydrolysis and the solution becomes alkaline, it is necessary to have a sufficient amount of buffer present to prevent any marked change in the hydrogen-ion concentration. In this investigation, a quantity of enzyme material has been taken which will generally produce less than 1 mg. of  $\text{NH}_3$  in 30 minutes. A concentration of 0.1 M phosphate buffer (prepared from  $\text{Na}_2\text{HPO}_4$  and  $\text{KH}_2\text{PO}_4$ ) was used, which when present in the enzyme substrate mixture was found to keep the hydrogen-ion concentration from increasing more than three-tenths of a pH unit.

The *recovery of ammonia with the aeration apparatus* was determined, using standard solutions of ammonium sulphate, and also using mash plus standard ammonium sulphate solutions. Although 90 per cent. of the ammonia was recovered after 30 minutes of aeration at room temperature, recovery was complete only after two hours of aeration. The presence of mashed tissue did not interfere with recovery.

**SAMPLING OF PLANT MATERIAL.**—Analyses were made on the basis of the fresh weight of plant organs or parts of organs. The organs were removed



from the plants in the morning, then weighed and analyzed immediately. For each analysis generally ten organs were selected from ten different plants. The tissues were ground and duplicate determinations were run on aliquots of the mash, including also a control for the free ammonia present in the mash. In selecting samples for analysis, an attempt was made to choose plants that were similar in number and length of leaves and internodes. Plants either more or less vigorous than the average were discarded.

### Discussion and conclusions

It is necessary to clarify the term "quantitative" in reference to enzyme determinations. An enzyme is detected only through the catalysis of a more or less specific reaction. This criterion is, however, not sufficient to establish the absence or the total quantity of enzyme, but merely the amount of "active" enzyme present.

An extensive review by LÖVGREN (14) of the inactivation of urease by various substances indicates that inactivation, in most instances, can readily be accounted for by a denaturation or coagulation of the protein. As for activation by specific substances, WALDSCHMIDT-LEITZ and STEIGERWALD (26) report that urease activation with glycine or hydrocyanic acid is observed only with crude enzyme preparations, and not with purified or crystalline enzymes. There is some evidence (2, 5, 7) that oxidation inactivates and reduction reactivates urease but the meaning of this is not clear.

The data at present available on crystalline hydrolytic enzymes, including urease, favor preponderantly the contention (24) that these enzymes are proteins containing in their make-up, and holding by chemical linkages, peculiar arrangements of atoms which constitute the active groups. Since urease can be detected only in its active state, that is, by the number of active groups with which the urea molecules can come in contact, it becomes important to examine under what conditions and to what extent these groups are available for action.

In the cells, proteins are present in various states of aggregation, some protein molecules being monomolecularly dispersed, others clustered into larger groups, and still others aggregated into ergastic protein materials such as the globoids, aleurone grains, and crystals. The protein urease likewise can be considered to be present in the cells in these various states of aggregation. GRABAR and REIGERT (6) have recently investigated the activity of urease as related to particle size. Using four different urease preparations they found, by means of membranes of graded porosity, that the particles of urease were in different states of aggregation. Crystalline urease in aqueous solution was the most homogeneous preparation examined, and had dimensions near those of serum globulin. On digestion of the urease preparations with trypsin it was found that those particles small enough to pass through pores 15  $\mu$  in diameter possessed no urease activity. Under the same

conditions serum albumin passed through pores 30 m $\mu$  in diameter but not through 9 m $\mu$  pores. Crystalline urease was the most active preparation. From these data, it appears that the activity of a urease preparation is at its maximum when the urease particles are of the dimensions of serum globulin particles (mol. wt. 150,000); presumably the urea molecules are now able to come into contact with all the available active groups of the urease particles. The work of KIRK and SUMNER (12) may be cited as evidence that the decrease of urease activity occurs with agglomeration of urease particles. These workers noted that the inhibiting effect of anti-urease (antibody) on urease consists, largely but not entirely, in decreasing the dispersion of urease.

From the above, it may be seen that when larger groups of urease molecules, as in the form of ergastic materials in the cell, are dispersed, an apparent synthesis may occur. If urease is decomposed proteolytically below a certain molecular weight there will be a loss of urease activity; but there may also be a decrease in urease activity or apparent decomposition when the enzyme becomes denatured, agglomerated, or stored in some form in which it is not readily dispersed. The urease that is detected is, in the main, in a highly dispersed condition. The data on the rate of inactivation of mash of jack bean leaf show an increase in urease activity during the first three hours after maceration, indicating that more of the active groups have become available than are being removed by autolysis or denaturization.

There is no way at present of determining the total quantity of enzyme present (16, 18). The methods used in this investigation have been chosen to determine as nearly as possible the relative amounts of available active groups present in the cells at the time of examination. In all instances, where comparisons could be made of the histological and the quantitative methods with the same tissue, it was found that the results obtained with both methods checked each other nicely. One may infer, therefore, that in the macerated tissue, the enzyme does not differ in activity to any extent from the enzyme in the cells of tissue sections. The experiments in which "whole" and "macerated" leaf tissues were compared showed that macerating caused no detectable inactivation of the enzyme. Further experiments (table III) indicate that urease inactivation within the first 30 minutes after crushing the tissue is slight or negligible; and this has been the length of time chosen for the enzyme to act on urea.

Although, with the cotyledonary tissue of soy bean, there is no increase in the rate of urea decomposition above 0.2 M urea concentration, this is not true for the tissue of the jack bean leaf. In this leaf tissue, there still appears to be an appreciable increase in the decomposition rate of urea even above a 2.1 M concentration. This may be another instance of making the active groups of urease available, since it is known that urea in high aqueous concentrations has a powerful solvent action on many proteins, and appears to split certain

protein molecules. Edestin, for example, is split into particles whose molecular weight is about one-fourth of that determined by SVEDBERG for edestin in neutral salt solutions. For convenience, a concentration of 0.2 M urea was used in these determinations. When different plant structures are to be compared as to total urease content, an error of 25 per cent. may arise because of an insufficient urea concentration; when the same tissues or plant structures are being compared, however, this error is eliminated. Fortunately, the differences of urease content of different structures are much greater than 25 per cent. so that this factor does not influence any of the conclusions that have been drawn from the data.

Although the limit of sensitivity of the quantitative method is 0.0001 U.U. per gram of fresh weight of tissue (where a urease unit, U.U., is that quantity of urease which will produce 1 mg. of ammonia per minute), the histological method is not as sensitive. From comparative determinations it has been found that the indicator method cannot detect urease in tissues having a lower activity than 0.02 U.U. per gram fresh weight.

By making certain assumptions,<sup>3</sup> it may be calculated that there are about 25 urease molecules contained in a cell, at the limits of sensitivity of the quantitative method. Since the indicator method is only one-two hundredth as sensitive as the quantitative method, at least 5000 urease molecules must be present in a cell in order to change the indicator color. It is therefore impossible to determine the positions of the enzyme in the cells by the indicator method unless the urease molecules are sharply localized in specific regions.

### Summary

1. Two methods for the determination of urease are described. The first, a histological method, depends on detecting the increase in alkalinity of the cells as urea is being hydrolyzed by the urease present in the cells. This increase in alkalinity is made evident either by the use of a suitable pH indicator or by the formation of a lake. The second, a quantitative method, depends on the determination of the ammonia produced when urea is hydrolyzed by the enzyme.

2. The following factors were considered in applying the histological method: The hydrogen-ion concentration of the tissue; the suitability of various indicators; the permeability of cells to indicator dyes and to urea; the buffer capacities of cells and reagents; the time factor for the method; and the preparation of tissue sections for analysis. In the lake method, reagents were chosen which would form insoluble and highly colored precipitates at the enzyme points.

<sup>3</sup> Assumptions: Dimensions of a parenchyma cell  $20 \times 50 \times 50 \mu$ ; density of cell 1.0; there is one active group per urease molecule; urease has a molecular weight of 150,000; 1 gm. of crystalline urease can produce 26,000 mg.  $\text{NH}_3$  per minute, which is the highest activity reported by SUMNER (23).

3. The following factors were considered in applying the quantitative method to various tissues: Effect of drying the tissues; effect of macerating the tissues; effect of various urea concentrations on the maximum urease activity; effect of the temperature; effect of the time; the rate of inactivation of urease; the effect of the hydrogen-ion concentration; the choice and concentration of the buffer; the recovery of ammonia by aeration; and the sampling of tissues for analysis.

4. A concept of urease activity, based on the number of available active groups of urease, is discussed.

The writer wishes to express his gratitude for the many helpful suggestions and the kindly criticisms of Professor F. G. GUSTAFSON throughout the course of this investigation.

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# A RECORDING POTENTIOMETER FOR USE IN PHYSIOLOGICAL INVESTIGATIONS

R. H. WALLACE

(WITH SEVEN FIGURES)

## Introduction

Some years ago during the process of developing a vacuum tube voltmeter it was found necessary to build a recorder that was adequate to test and check the voltmeter. The instrument described in this paper is one evolved from the early recorder during more than three years of experimentation.<sup>1</sup>

It was found upon investigating the many types of recorders on the market that none could be said to be adaptable enough or to have the numerous other characteristics required for use in physiological problems. For this reason the fundamental ideal kept constantly in mind during the development of this instrument was to make every part as universal and as adaptable as was possible without sacrificing dependability or accuracy.

## Characteristics of the instrument

### SENSITIVITY

Perhaps the first characteristic that we demand of an instrument is sensitivity. In this respect the one described below leaves little to be desired. As ordinarily adjusted the present instrument is activated by 0.1 microampere or, in terms of voltage, 0.1 millivolt. If, however, higher or lower sensitivity than this is desired it can be obtained. With maximum adjustment for sensitivity the instrument can be made to work well on 0.01 microampere. If, on the other hand, the potentials being measured are high, one can, by suitable shunts across the null galvanometer, tone the instrument down so that even tenths of volts are necessary to activate it.

### MILLIVOLTAGE RANGE OF BRIDGE

The one characteristic that is more inadequate than any other in most commercial recorders is the failure to equip them with a universal bridge. Only one or at most a few different scale values are given, and anything that is being measured which does not fit these arbitrary units must be modified by shunting or other devices to bring them within the scale of the instrument. In the present case this is entirely eliminated. The bucking voltage of the instrument is furnished by dry cells. If the potential which we are to measure does not exceed 1500 millivolts, ordinary flashlight dry

<sup>1</sup> The writer wishes to express his appreciation to JOHN L. REINARTZ for demonstrating the feasibility of this type of instrument.

cells are used, but if greater values than this are needed one can substitute the number of 45-volt "B" batteries needed.

Also by means of a suitable arrangement of resistances one can adjust the bridge so as to get any millivoltage from 3 to the full value of the battery. This is done by the mere turn of the required dial. In addition to this universal control of the millivoltage value of the bridge, one can likewise place the zero point of the bridge at any desired location between the two ends of the bridge. But even more important than this is the fact that the shifting of the zero for any point between the two ends of the bridge can be done without modifying the sensitivity for which the bridge is set. Below will be found the method whereby these adjustments are accomplished.

#### CALIBRATION

Calibration is made with the usual potentiometer. One simply decides the millivoltage in which he is interested and then sets up a potentiometer with this value. He then connects this to the input leads of the recorder and adjusts the sensitivity control resistance so that this millivoltage gives him the centimeter deflection on the recorder drum that he desires. He then adjusts the zero control dial so that the zero is where he wishes it. Further information on this point will be found below. Under ordinary conditions this calibration is good for months. If at any time the batteries begin to fail, the action of the instrument makes this immediately obvious. Ordinarily the batteries have shelf life; that is, they last just as long as if they were stored on the shelf—at least one year is reasonable. Obviously one can recheck the calibration in a few minutes at any time.

#### STABILITY

The instrument has functioned for months with no attention other than the replacement of the pilot bulb once in a while and other minor items of that sort. No fundamental element of the instrument has been replaced in a year of continuous use. The instrument even functions well when line voltage fluctuates from 100 to 120 volts. On one occasion when the instrument was being used as a light recorder illness prevented me from being in the laboratory for a period of two weeks. Nevertheless, when I did return, there were the complete records for the period.

#### LENGTH OF RECORD AND SPEED OF MOVEMENT OF PAPER

The drum can be made to turn once a minute, once an hour, or twice a day, depending upon the shaft of the clock that is used for attachment. For most purposes, however it is set to turn twice a day. Since the drum itself is 36 cm. in circumference, the speed of movement of the paper is 3 cm. per hour. A 150-foot roll of recording paper can be put in at one

time, which is a month's supply (day and night run) when the drum revolves twice daily.

#### AVAILABILITY AND COST OF THE INSTRUMENT

The instrument can be built by anyone handy with his hands and able to do simple wiring. A complete set<sup>2</sup> of five blueprints is available showing the full size and shape of all parts and the exact connections to be made. Two types of wiring schemes are given. One type, the ordinary schematic one of the radio engineer (fig. 2), that shows grids, cathodes, plates, etc., means something to those initiated into the realm of the radio but it means little to the rest of us. The other wiring scheme is purely pictorial and shows the exact size, spacing, shape, and connection for all parts (figs. 4, 5). The total cost, if one purchases all the mechanical parts ready-made but does the wiring himself, is about \$125. This price, of course, includes all educational discounts. Anyone who understands the radio circuit type of wiring should complete that part in a day. The adjustment may require somewhat longer.

#### How the instrument works

Fundamentally this instrument is a self-balancing Wheatstone bridge of the null-indicator type. That is, a voltage is put across the two ends of a resistance wire. If one connects low resistance wire to one end of this system and then touches the other end of the wire to any point along the resistance wire, a meter in series with the low resistance wire will deflect, showing that there is a voltage along the first wire. This voltage constitutes the bucking voltage against which the unknown, which is being measured, acts. The rest of the instrument consists merely of the various elements necessary to move the traveling arm back and forth as the voltage of the unknown changes so that at all times the voltage on the resistance wire balances to 0.1 millivolt the voltage of the unknown. When this condition is attained the instrument is in balance and the meter in series with the low resistance wire shows no deflection; that is, it is null.

#### THE BRIDGE

Let us now refer to figure 1A to see how the universal bridge works. Assume that the wire  $AB$  is the bridge of the recorder and that  $R$  is the potentiometer controlling the sensitivity of the bridge. Then if  $U$  is a 1.5-volt (1500 mv.) dry cell and  $R$  is turned to no resistance, that is,  $t$  is up

<sup>2</sup> This complete set together with list of parts needed, catalog numbers, addresses of places of purchase, and all other information for the construction and adjusting of the instrument can be obtained for \$1.00 by writing to R. H. Wallace, Storrs, Conn. All figures used in this paper as well as the originals from which the blueprints are made were drawn by H. E. HILL.



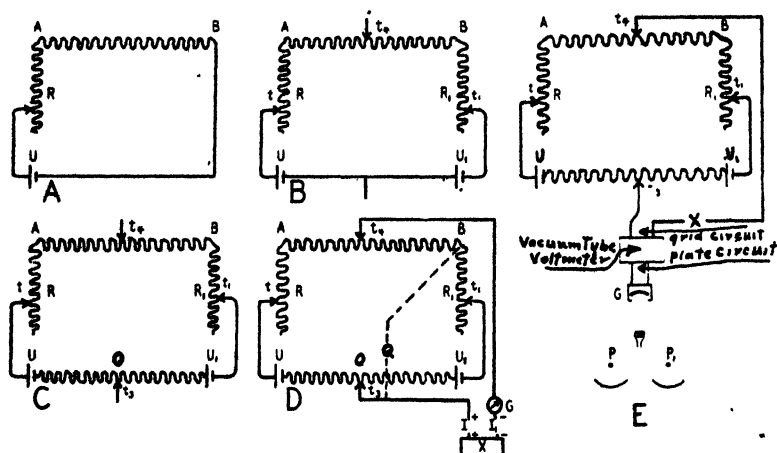


FIG. 1. Schematic wiring diagrams of bridge and method of connecting the bridge to a vacuum tube voltmeter. A, basic diagram of circuit showing how the voltage on bridge  $AB$  can be controlled by the adjustment of the variable resistance  $R$ . B, circuit similar to A, except resistance  $R_1$  and battery  $U_1$  have been added making circuit balanced with zero between  $A$  and  $B$ . C, same circuit as in A and B with resistance  $O$  added to control the position of zero without modifying the sensitivity of the bridge. D, complete schematic diagram of the bridge of the recorder showing the method of connecting the null-control galvanometer and the unknown  $X$ . E, necessary modification of the bridge connections when the recorder is used with a vacuum tube voltmeter. For details regarding the bridge circuit and how it works see text.

to point  $A$ , then the full voltage of  $U$  or 1500 millivolts will be across the bridge from  $A$  to  $B$ . The resistance of wire  $AB$  is 500 ohms and that of  $R$  is 25,000 ohms, so that the ratio of the two is 1:51. If then  $R$  is turned to full resistance (25,000 ohms), the voltage between  $A$  and  $B$  will divide up now in the ratio of this resistance or 1 to 51 so that the voltage now present from  $A$  to  $B$  will be  $1500 \div 51$  or 29.4 millivolts. With this combination the bridge can be set for any value between 29.4 and 1500 millivolts, depending upon how much is put in from resistance  $R$ . Automatically, however, zero voltage is at  $B$ , since there is no resistance between  $B$  and the terminal of the battery. This zero point cannot be shifted.

Suppose (fig. 1B) we now add another resistance ( $R_1$ ) which is identical to  $R$ . With  $R$  and  $R_1$  now set for no resistance the voltage across  $AB$  becomes double that above because  $U_1$  has been added and it is now 3000 millivolts. On the other hand, if both  $R$  and  $R_1$  are set for full resistance, the voltage across  $AB$  will divide as 1 is to 101, since the ratio of resistance is now 500:50,500. The total voltage between  $A$  and  $B$  will therefore be 29.7 millivolts. Now, however, the zero point has shifted up from  $B$  to some point near the middle of the bridge so that point  $A$  is 14.85 millivolts plus with respect to it while point  $B$  is 14.85 millivolts minus to it. The

zero point can be shifted back and forth by changing the resistance value of  $R$  or  $R_1$ , but one will be modifying at the same time the sensitivity of the bridge.

Suppose we now add a third resistance ( $O$ , fig. 1C), which is equal to or slightly greater than the resistance of  $AB$ . If this resistance is set with the traveling arm exactly in the middle of its resistance wire then the zero point on  $AB$  will be unchanged. If it is moved toward  $U$ , however the zero on the bridge will automatically shift an equal distance toward  $B$ ; while if it is moved toward  $U_1$ , the zero will move toward  $A$ . Since the resistance of  $O$  is equal to or greater than that of  $AB$ , therefore the zero can be shifted to any point between  $A$  and  $B$ . But notice this also; as you add more resistance to the  $U$ -side of the resistance  $O$  you take it away from the  $U_1$ -side. Consequently, the total resistance in the circuit between the traveling arm  $t_4$  of the recorder bridge and  $t_3$  of the zero adjusting potentiometer remains exactly the same. The zero can be shifted but the sensitivity is unchanged.

Reference to the dotted line in figure 1D will show another very valuable addition to the universality of the bridge. If one is measuring something which can drop to zero voltage, but never change sign, then, one can close switch  $Q$  and use the new bridge combination. When  $Q$  is closed, battery  $U_1$  and resistance  $R_1$  are shorted off the bridge  $AB$ . They are shorted on themselves, but this does no harm since resistance  $R_1$  is 25,000 ohms and therefore insures shelf life for battery  $U_1$ . The bridge has now become a simple one like that in figure 1A, except that resistance  $O$  is in the circuit. The attachment of the wire from  $Q$  to the moveable arm  $t_3$ , rather than directly to battery  $U$ , enables one to set zero for any point between the two ends of the bridge the same as in figure 1B above. This setting of zero entails modification of the sensitivity of the bridge, however, so that one must obtain the combination of zero and sensitivity of bridge by adjusting both  $R$  and  $O$ . The value of this zero adjustment will be discussed below.

Another valuable characteristic of this new bridge combination is the increased sensitivity of the null point which it gives. Thus, if one examines figure 1D he will see that the circuit from the unknown ( $X$ ) must be completed through the zero-adjusting arm  $t_3$  and the traveling-arm  $t_4$  of the bridge. Between these two points we have  $R$  and  $R_1$  in parallel which means that they act as one resistance of 12,500 in series. Also in series with this 12,500 ohms will be one-half of the total resistance (assuming  $t_3$  and  $t_4$  are on center) of bridge  $AB$  and resistance  $O$ , since they likewise are in parallel. We may then have as much as 13,000 ohms in series with the galvanometer when  $Q$  is open. On the other hand, when  $Q$  is closed the maximum is 1000 ohms. Since the null-control galvanometer itself has 1000 ohms resistance, this additional 1000 ohms will reduce its deflection per unit of voltage to just one-half when  $Q$  is closed. When  $Q$  is open, on the other

hand, it will be reduced to  $1/13$ . The instrument has so much sensitivity that even this loss is not important for most measurements, but in some cases this additional sensitivity is quite valuable.

Figure 1E shows the slight modifications in the wiring when the recorder is to be used with a vacuum tube voltmeter. The null-control galvanometer is disconnected from the bridge circuit and hooked directly in parallel with the plate circuit of the vacuum tube voltmeter. The unknown remains connected as in figure 1D, and the grid circuit of the voltmeter is connected directly in series with it.

We are now ready to complete the null connections (fig. 1D). If we now connect an unknown ( $X$ ) to the inputs  $I$  and  $I_1$ , so that polarity bucks that from the bridge, the galvanometer ( $G$ ) will deflect. If some suitable mechanism is activated by the deflection of  $G$  which can cause traveling arm  $t_1$  to move, then more or less voltage will be put into the circuit, depending upon which is required. When the voltage from the bridge exactly equals that from the unknown, then  $G$  will no longer deflect and the mechanism will be in the null condition. This mechanism for maintaining null constitutes most of the remaining story of the apparatus.

#### NULL-ACTIVATING DEVICE

**ELECTRICAL ARRANGEMENTS.**—Figure 2 gives the schematic wiring diagram of the entire mechanism that maintains the galvanometer on null. The galvanometer ( $G$ ) is placed directly in front of the pilot bulb ( $F_1$ ) and also in front of the two symmetrically placed photoelectric cells ( $P$  and  $P_1$ ). These photocells are attached to the control grids of the two mercury vapor

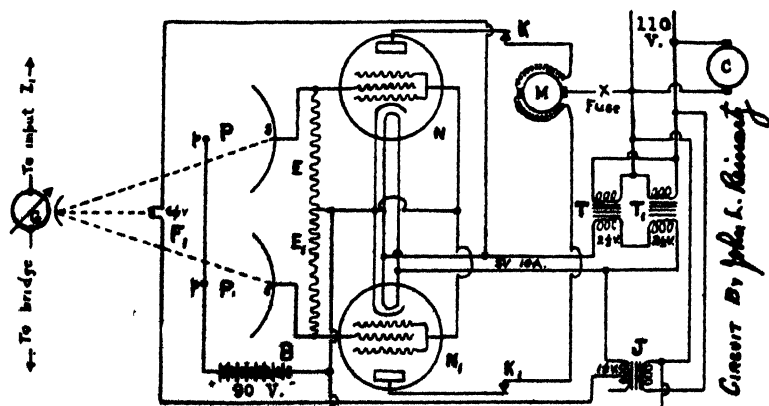


FIG. 2. Schematic wiring diagram showing all electrical connections necessary in the automatically maintained null.  $G$ , null-control galvanometer with mirror;  $F_1$ , pilot bulb;  $P$ ,  $P_1$ , photo-electric cells;  $p$ ,  $s$ , pin and shield of photo-cells;  $E$ ,  $E_1$ , grid leaks;  $N$ ,  $N_1$ , mercury vapor tubes;  $K$ ,  $K_1$ , limit switches;  $M$ , reversible motor;  $X$ , fuse;  $T$ ,  $T_1$ , and  $J$ , transformers;  $C$ , electric clock to turn drum. For further details see text.

tubes ( $N$  and  $N_1$ ) with grid leaks ( $E$  and  $E_1$ ) suitable for these tubes. The plates of these mercury vapor tubes are connected through the limit switches ( $K$  and  $K_1$ ) to the split fields of the reversible motor ( $M$ ). The twin transformers ( $T$  and  $T_1$ ) supply the 5-volt 10-ampere filament supply to the mercury vapor tubes. The supplementary transformer ( $J$ ) is connected in series with these twin transformers to increase the voltage to  $6\frac{1}{2}$  volts to supply the pilot bulb ( $F_1$ ).  $C$  represents the electric clock that drives the recording drum.

When the unknown is exactly balanced by the voltage on the bridge, the light from the pilot bulb ( $F_1$ ) hits the mirror of the galvanometer ( $G$ ) and is reflected back between the photocells ( $P$  and  $P_1$ ), the mercury vapor tubes are not activated, and the motor carrying the travelling arm on the bridge does not run. When the circuit is unbalanced, however, then the light may reflect back and hit the photocell ( $P$ ). This causes the shield ( $s$ ) to become plus and the voltage from the batteries ( $B$ ) is allowed to pass and put a plus charge on the control grid of the mercury-vapor tube ( $N$ ). This in turn allows the current to flow through the tube from the 110-volt circuit to one field of the motor ( $M$ ), causing the motor to run in one direction. If, on the other hand, the light is reflected on the tube ( $P_1$ ), the same series of events occur for that side of the circuit and the motor runs in the reverse direction. The only connection between the bridge circuit and this activating mechanism circuit is the beam of light reflected back from the galvanometer mirror. In the early models of this recorder relays were used instead of the mercury-vapor tubes, but since they continually gave trouble they were eliminated.

The radio tube in its simplest form consists essentially of a cathode, a grid, and a plate. This tube is connected into a circuit in such a manner that current tends to flow from the cathode, which is minus, to the plate, which is plus. This flow can take place only when the grid, which is between the cathode and the plate, is likewise plus. A radio tube can therefore be said to be an electronic relay. Most tubes allow only a few milliamperes of current to pass. The mercury vapor tube varies from these in being able to let many amperes through. The type used in this circuit will pass 4.5 amperes at 1000 volts. This gives a current-carrying capacity many times that required for the 1/80 H. P. motor used in this instrument.

Reference to figures 2 and 5 will show limit switches ( $K$  and  $K_1$ ). These are put in the plate leads from the mercury-vapor tubes and the fields of the motor to cut the motor out in case the traveling arm tries to overrun at the limit of its run. This eliminates any possibility of the instrument being damaged. Likewise it will be noted that at  $X$  there is a fuse in the common lead to the fields of the motor. This is to prevent damage in case both sides of the circuit are activated at the same time and the motor tries

to go in both directions at once. It will be noted that the clock for driving the drum is connected directly in parallel on the 110-volt line. In figures 4 and 5 are given the self-explanatory pictorial diagrams of the spacing of parts and wire connections.

**OPTICAL ARRANGEMENTS.**—Figure 3 gives a section through the instrument showing the exact vertical spacial relationship between the various

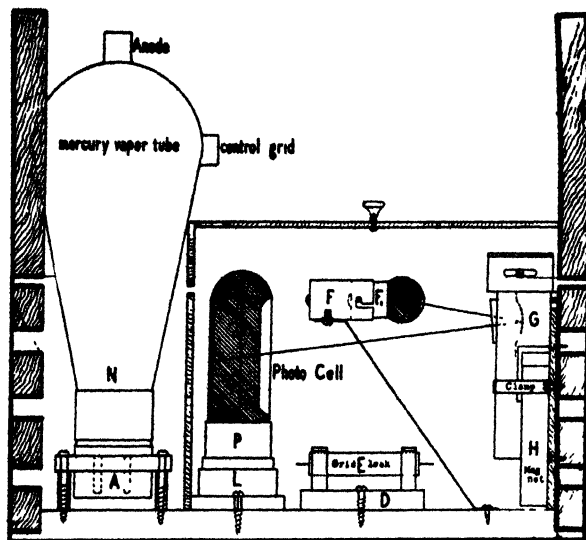
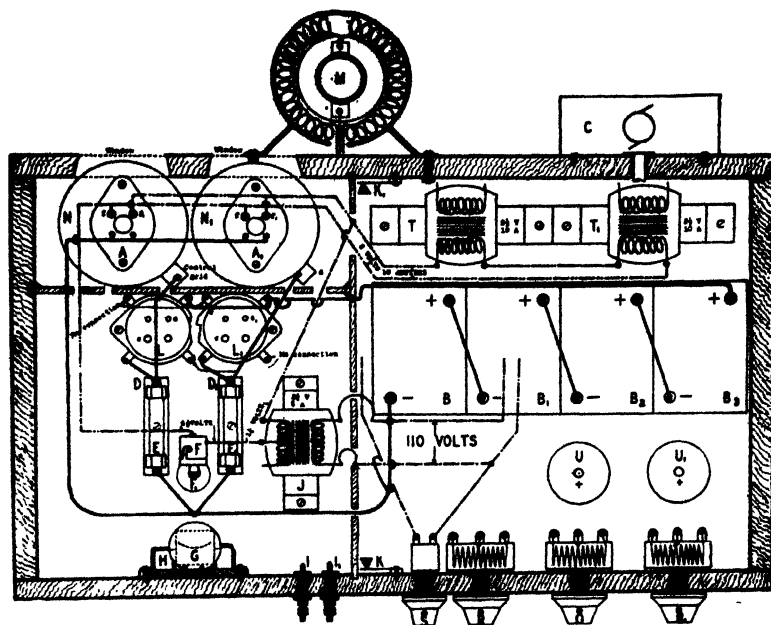


FIG. 3. Section through instrument showing exact spacial relationship of all parts. In this figure, and those following, these parts are shown in their true shapes and relative sizes.

parts. These relations must be followed very closely to obtain a workable optical arrangement. The shading on the pilot bulb ( $F_1$ ) and the photo-cell represent photographer's opaque which is used to mask out the excess light. Figure 4 shows the exact horizontal spacial relationship of all these parts.

**MECHANICAL ARRANGEMENTS.**—Figure 6 shows the motor with its reducing gear, the screw rod carrying the traveling arm, and the spacing and mode of attachment of the drum. It will be noted that opposite the traveling arm of the bridge there is another arm which moves along with it that is at the same time tracing the record on the drum. The recording paper is a wax-covered paper which requires no ink but merely pressure from a writing point to give a record. Figure 7 shows the spacing of parts on the front panel of the instrument. This spacing must be followed closely to enable one to get in all the parts as shown. The roll of paper is here shown as a full-sized roll of 150 feet.



null-maintaining mechanism.

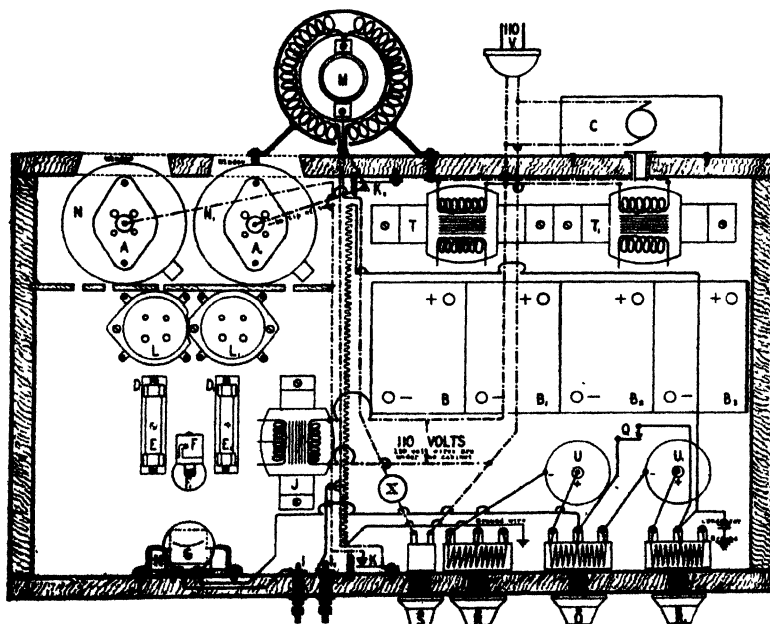


FIG. 5. Diagram showing the 110-volt connections of the null-maintaining mechanism and the wiring of the bridge.



The writer will not attempt to give in this paper the numerous details of construction and manipulation of the recorder. These will be found, however, in full detail on the blueprints or in the supplementary information enclosed with them. It may be said, however, that while this additional information greatly simplifies the work, nevertheless, anyone familiar with amateur radio construction can with no difficulty construct an instrument from the information here given.

A double recorder can likewise be constructed from these parts with only very minor modifications. This double recorder is made of two entirely separate but identical bridges and null-activating mechanisms inclosed in one cabinet and writing on one drum. The ground plan of this instrument is simply the plan as shown in figures 4 and 5, with the mirror image of this plan used on the other side of the drum. This instrument has the same fundamental characteristics as the single recorder. The bridge arrangement controlled by the switch (*Q*) gives one complete control of the location of zero. This is a very valuable characteristic to have in a duplex recorder. Thus one can set the two unknowns under test for equal centimeter deflection on the drum with their zeros on the same base line. Furthermore, one zero can be 1 cm. below the other, or there can be any other combination desired. One can even have one base line at one end of the drum and the other at the other end and have the curves move toward each other, or have them in the center of the drum and measure in opposite directions. Each of these combinations has its special suitability for certain materials under measure. The quality of work which this instrument can do will be amply shown in a study of light sampling to appear in an early issue of this journal.

### Application of the instrument

#### RECORDING POTENTIOMETER OR MILLIVOLTMETER

This instrument can be used in experiments involving a great number of physiological problems because of the adaptability of the various parts. It has worked excellently for testing the stability of vacuum tube voltmeters and for recording voltages being measured by means of a vacuum tube voltmeter. It has also been used on hydrogen ion and electrometric titration. The chief application so far made of it has been the recording and study of methods of measuring light. It is peculiarly adapted to this use because of its universality. Some preliminary tests have been made of it with thermocouples for temperature measurements, and it seems equally well suited for this work. A study of the characteristics of the instrument will indicate to anyone many other applications.



## AUTOMATICALLY MAINTAINED NULL

A very valuable application of this instrument is that of an automatically maintained null device accurate to 0.1 microampere or 0.1 millivolt. That is, one can use it to measure pH or to maintain pH. If one sets the traveling arm of the bridge for some pH he desires and then disconnects the anode leads of the two mercury vapor tubes from the motor and connects them instead to an acid- and an alkali-adding device, the pH value can then be maintained with very great accuracy. The mercury vapor tubes are capable of running an  $\frac{1}{8}$ -horse power motor which gives some idea of the power available for maintaining any type of null desired.

The writer does not consider the present instrument perfect, and therefore welcomes all criticisms and suggestions for its improvement. In this manner, although the instrument may be far from perfect at present, we can hope finally to develop an instrument ideally suited to the very exacting requirements of physiological work. This instrument had its inception in the study of vacuum tube voltmeter stability. It is not and, in the very nature of the many problems involved in its evolution, cannot be the work of one person. It is therefore with the very greatest pleasure that I express my indebtedness to E. L. KELLY, D. G. STEELE, JOHN L. REINARTZ and L. B. CRANDALL for their invaluable assistance. That which this instrument is, it is through the cooperative efforts of us all.

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# CONTRIBUTION TO THE KNOWLEDGE OF PLANT PHENOLASES<sup>1</sup>

RUDOLF M. SAMISCH

(WITH ONE FIGURE)

## Introduction

Respiratory chromogens are thought by PALLADIN and his coworkers (16, 17) to play an important rôle in plant respiration. Dihydroxy and trihydroxy phenols are found among these chromogens. They are oxidized to quinones by molecular oxygen in alkaline solution. In acid solution, a similar oxidation occurs in the presence of oxygen and plant phenolases. The possible rôle of phenolase in plant respiration has been questioned, because attempts to correlate activity with rate of respiration have failed; furthermore, phenolases could not be found in a large number of plants. It should, however, be born in mind that a correlation between phenolase activity and rate of respiration can be expected only if the enzyme is the limiting factor in the system. That this is not the case can be seen from the fact that the addition of phenolic compounds to a plant extract known to contain phenolase is followed by the development of colored products. In other words, the amount of enzyme present is larger than that required for the oxidation of a plant chromogen normally present and the chromogen appears to be the limiting factor. The fact that phenolases have not so far been demonstrated in a number of plants may be due to the fact that the possibility of specificity among phenolases such as is known to occur among other enzymes has been ignored. Thus the reagents used for the determination of their presence may have contained reactive groupings that are attacked by some phenolases but not by others. That such a condition prevails will be shown in this paper, in which observations are reported which appear to indicate the existence of three phenolases, specific for the ortho-, meta- and para-hydroxy grouping respectively.

## Methods

The plant extracts were prepared from frozen material by extraction with 1 per cent. sodium bicarbonate solution. The extracts were immediately neutralized with glacial acetic acid and saturated with toluol. Phenolase activity was measured by observing manometrically the rate of oxygen absorption by the extract-substrate mixture in a Barcroft-Warburg respirometer. The reaction took place under oxygen at 25° C. in a

<sup>1</sup> From the Division of Fruit Products and the Division of Plant Nutrition, University of California, Berkeley.

medium buffered with an acetate mixture. None of the extracts showed a measurable oxygen absorption in the absence of a suitable poly-phenol. The details of the method have been discussed elsewhere (19).

### Experimentation

The catalyzing power of different plant extracts was tested on various phenolic substrates at varying concentrations and pH. The extracts from fruits of apricot (var. Royal) and avocado (var. Spinx) oxidized catechol very rapidly and pyrogallol only slowly. The ratio for avocado was about 6:1<sup>2</sup>. Avocado extract oxidized phloroglucinol at an exceedingly slow rate, while apricot extract caused observable effect. Neither avocado nor apricot extracts oxidized phenol, resorcinol, quinol, or tryosine, although potato extracts oxidized tryosine at a rapid rate. The avocado and apricot extracts were used with resorcinol between 0.1 and 1 M concentration from pH 3.9 to 7.1, and with quinol at 0.15 and 0.3 M concentration between pH 3.9 and 7.1. The tyrosine was used only at pH 6.5.

An extract of lemon leaves (var. Eureka) was found to oxidize phloroglucinol very rapidly, but it oxidized resorcinol very slowly (pH 6.2, conc. 0.5 M). Catechol and pyrogallol were oxidized at a considerably slower rate than phloroglucinol (table I). No oxidation of phenol or tyrosine was obtained. The ratio of the rate of phloroglucinol oxidation to that of catechol was about 11:1. A rather interesting phenomenon observed was the action of catechol upon the phloroglucinol-oxidase system. Catechol inhibited the oxidation of phloroglucinol (table I).

TABLE I

EFFECT OF CATECHOL UPON THE RATE OF OXYGEN ABSORPTION BY LEMON LEAF EXTRACT IN THE PRESENCE OF PHLOROGLUCINOL AT pH 6.2 AFTER 20 MINUTES (IN MG.  $\times 10^4$ )

SUBSTRATE	0.0125 M PHLOROGLUCINOL	0.0125 M PHLOROGLUCINOL 0.05 M CATECHOL	0.05 M CATECHOL
Enzyme and substrate	315	166	138
Substrate blank	0	90	110
Enzyme and substrate corrected for blank	315	76	28

After exposure to oxygen without the enzyme the phloroglucinol remained almost colorless although having a greenish-yellow tinge; the catechol had browned; but a mixture of the two became green. When the enzyme was

<sup>2</sup> Unless stated otherwise, this ratio refers to absorption of oxygen under optimum substrate concentration.

present the mixture of the two substrates turned greenish black. The mixture of catechol and phloroglucinol prepared in a test-tube prior to the determination remained almost colorless in absence of the buffer. When auto-oxidation was hastened by raising the pH the dark green pigment developed. The amounts of absorption by the substrate blanks (table I) seem to indicate that the presence of phloroglucinol only slightly inhibited the auto-oxidation of catechol. From this we may infer that the reactive OH groups of the catechol were not involved in the reaction causing the formation of the green pigment associated with the inhibition of enzyme action of phloroglucinol in the presence of catechol. A possible explanation of the inhibition of phloroglucinol oxidation by catechol would seem to lie in a reaction of the phloroglucinol with the oxidation product of the catechol.

The extracts from young pear leaf (var. Bartlett) very rapidly oxidized quinol. Catechol was oxidized at about one-fourth the rate of quinol (table II), and pyrogallol still more slowly. Resorcinol, phloroglucinol, phenol, and tyrosine were not oxidized to a measurable extent.

The various extracts had different properties in respect to their power of oxidizing different phenolic groupings. Apricot and avocado extracts oxidize most rapidly the ortho-hydroxy-phenol, lemon extract the meta-hydroxy-grouping, and pear the para-compounds. More than one such group was attacked by most extracts. On the assumption that more than one enzyme was present, attempts were made to separate them. The enzymes were fractionally precipitated by alcohol, filtered, dried, and redissolved in water. The relative activity of the precipitate for different substrates was tested. However, no significant differences could be obtained because of the great differences in the rate of catalysis of any one plant extract toward any two substrates. This necessitated correspondingly large dilution of the stronger one, thus increasing the error considerably. The enzymes in avocado, apricot, and lemon could be precipitated with 75 per cent. alcohol while the enzymes in the pear could even be precipitated with 45 per cent. alcohol.

It has been shown (21) that the catalytic power of apricot extract is destroyed by exposure for only 1 hour at 70° C. Extracts of pear leaf and lemon leaf (without buffer) were brought to boiling and then immersed in boiling water for one-half hour. Table II shows the effect produced by boiling. While the power to oxidize catechol was lost by the lemon leaf extract, it was still able to slowly oxidize phloroglucinol. The power to catalyze the oxidation of quinol had been completely retained by the pear leaf extract while it had lost most of its action on catechol. Thus in the same extract one catalytic power could be destroyed, while the other remained unharmed, which lends strong support to the theory that we are dealing with more than one enzyme.

TABLE II

EFFECT UPON PHENOLASE ACTIVITY OF HEATING FOR ONE-HALF HOUR AT 100° C.

SOURCE OF EXTRACT	SUBSTRATE	pH*	O <sub>2</sub> × 10 <sup>3</sup> MG. ABSORBED† AFTER 10 MINUTES	
			UNHEATED	HEATED
Lemon	{ 0.5 M catechol .....	5.6	97	0
	{ 0.0125 M phloroglucinol .....	6.3	2670	47
Pear	{ 0.5 M catechol .....	5.6	56	8
	{ 0.05 M quinol .....	6.0	236	237

\* pH at which oxygen absorption was determined (buffer added after heat exposure).

† All values corrected for auto-oxidation.

These extracts were purified by precipitation and reprecipitation with three times their volume of alcohol, and their pH-activity curves (fig. 1) and Michaelis constant were determined. The activities, as influenced by pH, are plotted here on different scales in order to be able to show the curves on one graph. In each case, however, the intersection of the ordinate on the abscissa is used as the zero point. While the apricot-catechol and the lemon-phloroglucinol curves show definite optima in the acid range, no optima occur in the pH range studied for the pear-quinol and the avocado-pyrogallol systems. No measurements were taken above pH 6.2 because of the greatly increased auto-oxidation of the substrate. It has been pointed out (20) that the catechol system, when obtained from different sources, shows an optimum pH, which is presumably due to the accompanying specific colloids as it differs for different plants. Further work would probably establish a similar relative pH relationship between the various enzyme-substrate systems in different plants.

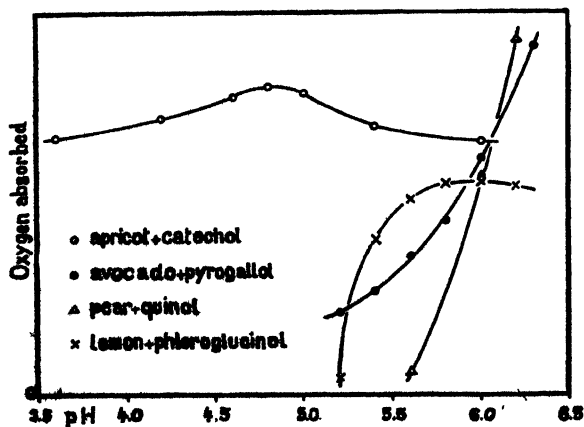


FIG. 1. pH-activity curves of different enzyme-substrate systems.

The Michaelis constant was determined by plotting substrate-concentration-activity curves and finding by this graphic method the concentration at which 50 per cent. activity was obtained. The results are shown in table IV.

TABLE IV  
MICHAELIS CONSTANT OF DIFFERENT PHENOLASES

SUBSTRATE	CATECHOL	PYROGALLOL	PHLOROGLUCINOL	QUINOL
Source of extract	Avocado Apricot	Avocado	Lemon	Pear
Michaelis constant	$7 \times 10^{-2}$	$2-3 \times 10^{-2}$	$6 \times 10^{-3}$	$1-2 \times 10^{-2}$

These values, ranging between  $6 \times 10^{-3}$  and  $1 \times 10^{-2}$ , differ in their order of magnitude from those reported in the literature. Thus WARBURG (24) found oxygenase of yeast to have a  $K_M$  of  $0.5 \times 10^{-7}$  M oxygen, and WILLSTÄTTER and WEBER (26) reported a  $K_M = 6 \times 10^{-6}$  M leucomalachite green for peroxidase of horse-radish. These and similar values for other oxidases caused HALDANE (8) to suggest that the low constant of oxidases may prove to be a fundamental distinction from hydrolytic enzymes. EULER and LAURIN (5) have shown that purification does not influence the Michaelis constant. The different values which they obtained for the Michaelis constant of saccharase from various races of yeast seem to be due to the presence of inactive enzymes which have retained their affinity for the substrate but do not dissociate from it. Since this would tend to produce lower values rather than higher ones, this factor does not seem to enter into the divergence which is reported here from the results of other workers.

### Discussion

The observations reported in this paper tend to indicate that phenolases from different sources may be of a different nature. This expresses itself primarily in their specificity towards definite groupings. BERTRAND (1) compared the relative rate of oxidation of phenols in the presence of lactase and found the rate of oxidation was in the order: quinol > catechol > resorcinol. More recently BUNZEL (2), working with various organs of the potato plant, came to the conclusion that the order of oxidation by oxidase was: para > ortho > meta. On the other hand, he found later (3) that this relation may not hold true in other plants and that the relative order of ease of oxidation of different phenols may vary in different plants. In no case was the meta position found to be easily oxidized.

The writer's plant material has the advantage that in all cases tyrosinase was absent, and that it was not measurable by the test-methods employed. This enzyme, which OKUYAMA (14) has shown to be essentially

a dehydrogenase, may be responsible for the high rate of oxidation of the para grouping reported in some of the data. In each of the three types of material under discussion a di- or trihydroxy phenol with a different relative position of the hydroxy groups in the phenol ring was preferentially oxidized by the enzyme. We prefer to speak of relative rates rather than of absence of the oxidation of certain groupings because the oxidation may have taken place at such a slow rate that it remained unnoticed under the conditions of the experiment.

It is, however, of interest to note in connection with the predominant rôle which ONSLOW (15) places upon catechol compounds, that the ortho grouping was more or less oxidized by the extracts in all three cases. Furthermore the oxidation of pyrogallol was slower than that of catechol, which shows that a group in a different position than those directly attacked may influence the reaction.

These findings give rise to the following question: Do the preparations represent different enzymes, or is there fundamentally one enzyme with its activity modified by various plant colloids? BUNZEL (3), who did not have quite as striking a case with his material, thought that the modification in the relative rate of oxidation of several substrates by various plants was due only to differences in the colloidal substances accompanying the enzyme. He stated that this contention was supported by the fact that all organs of the same plant behaved alike, while if he had dealt with a mixture of enzymes, they would have been expected to differ in their behavior. Yet his data do not seem to bear out his conclusion inasmuch as various organs apparently show such differences.

Of the three types of extracts discussed in this paper, the pear-leaf extract can be readily singled out from the other two. Para-phenolase of pear leaves is heat stable while the accompanying ortho-phenolase is easily destroyed by heat. Indeed, the heat stability of the former would lead one to believe that it is an inorganic catalyst. Heat stable oxidases have been reported in alfalfa (4), tobacco (6), and mushroom (25). Furthermore a major part of this para-phenolase was precipitated by the addition of an equal volume of alcohol to the solution, while the other phenolases necessitated the use of a higher concentration of alcohol for precipitation.

The enzymes of the lemon-leaf extract could not be separated by fractional precipitation with alcohol. Indeed, the fact that catechol greatly retarded the oxidation of phloroglucinol by lemon-leaf extract may provide an explanation for the reported very weak oxidation of the meta grouping found in the case of avocado. Avocado reacts positively to the ferric chloride test used by ONSLOW (15) for the identification of catechol compounds. Citrus was classed by her among "peroxidase" plants for the lack of response to this test. This would seem to suggest that we may

be dealing with the same enzyme, the oxidation of the meta grouping in avocado and other "catechol plants" being inhibited by the presence of an ortho-phenol. However, it is difficult to see how such a small concentration of ortho-phenol, as naturally occurs in the plant, should completely inhibit meta oxidation as, *e.g.*, in apricot. While much higher concentrations used *in vitro* for lemon extract fail to stop it completely. The inhibition of meta oxidation by catechol cannot be explained on the basis of a greater combining power of the enzyme with catechol as compared with phloroglucinol because the Michaelis constant of the meta oxidation is only half that of the ortho reaction, showing that the meta grouping is more strongly attached to the enzyme.

Thus we found an oxidase able to oxidize ortho-phenols in apricot and avocado, plants containing tannins which gave a test for the catechol grouping. The concentrated tannin extracts of apricot (20) and solutions of commercial tannins were rapidly oxidized by the diluted oxidase of apricot and avocado. Lemon-leaf extract oxidized phloroglucinol preferentially, while in common with other citrus plants it is known to contain hesperidin, a glucoside of a meta-phenol. A para-phenolase was expected in pear leaves, because they are known to contain arbutin, a glucoside of quinol, and it was actually found to be present in large amounts. Apparently different specific oxidases and their proper substrates are present in different plants.

The question need be raised, as to why the oxidation of these phenolic chromogens does not take place in so many plant tissues unless they are injured, in view of the fact that both substrate and enzymes are present in the vacuolar sap (18). This failure of pigment formation in the living cell, assuming the amount of the enzyme or any additional oxidative agent and the pH of the cells to be constant, may be due to one or more of the following mechanisms: (1) the rate of oxygen supply, (2) the amount and availability of the substrate present, or (3) the amount of an additional reducing substance present. The work of STEWARD (21) seems to indicate that oxidation and respiration of plant cells at cut surfaces may be limited by oxygen supply and it would seem that the influx of oxygen may play a major rôle in the darkening of cells beneath the cut surfaces of solid tissues. The fact that the substrate may be the limiting factor has been illustrated recently by KERTESZ (11). He described the oxidase system of the "Sunbeam" peach, and he believed that the enzyme is apparently not different from that of other stone fruits, but that tannin is almost absent. This variety of peach will not discolor upon injury. In addition to a difference in quantity of the substrate there may also be a modification of its availability. Thus LLOYD (12) reports that for various plants tannin may be held at different stages of growth within a gel of cellulose-like substance which may prevent its color reaction with iron salts. A complex



formation of hesperidin, in which the glucoside is surrounded by sugar molecules, has been mentioned by HALL (10). These colloidal formations may very well form systems which modify the rate of oxidation in the plant cell by limiting the available free substrate. The presence and activity of hydrolytic enzymes forms a very important mechanism to make the active grouping available for the oxidative system if it is bound, *e.g.*, as glucoside.

A third factor to be considered is the presence of a reducing substance providing for the reversibility of the system. SZENT-GYÖRGYI (22) showed that in the potato ascorbic acid could play this rôle. The writer observed the effect of ascorbic acid upon the systems discussed in this paper and found that with all substrates formation of a visible pigment was prevented in the presence of ascorbic acid, while the uptake of oxygen continued. The oxidation of ascorbic acid could be followed in all cases by means of iodine titration. Furthermore, if the pigment had been produced by oxidation of the phenol in alkaline solution, it could be reduced to the leucoform by ascorbic acid. Further evidence of this possible rôle of ascorbic acid in pigment formation is furnished by MORGAN, FIELD, and NICHOLS (13), who found that apricots, which had darkened, had lost vitamin C, and by JOSLYN, MARSH, and MORGAN (10) who showed that darkening of orange juice would take place only after the amount of ascorbic acid had sunk to a low level. Reducing substances, other than vitamin C, may possibly prevent the formation of the respiratory pigments in plant cells. An enzymatic oxidation of the products obtained by alkaline hydrolysis of sugar has been reported by GUTHRIE (7). It would seem probable that the unstable forms of sugar, as they occur in the plant, are able to reduce the quinones, which are formed by the action of oxidases on phenols, or that the oxidized ascorbic acid may, in turn, oxidize other reducing substances as suggested by SZENT-GYÖRGYI (23). Such a system may form an important part of the respiratory system in the plant.

### Summary

1. The catalytic action of a number of plant extracts upon the oxidation of phenols was tested in a Barcroft-Warburg respirometer.

2. All extracts catalyzed the oxidation of more than one phenol, but preferential oxidation was shown by the various extracts for different phenols.

3. The ortho-phenolase from the fruits of avocado and apricot, which oxidized catechol rapidly and pyrogallol less rapidly, was inactivated by heating at relatively low temperatures. Meta-phenolase from lemon leaves, which oxidized phloroglucinol rapidly and resorcinol exceedingly slowly, was somewhat more heat resistant. Para-phenolase of pear leaf oxidized quinol and was heat stable.

4. The pH-activity curves and the Michaelis constants for these enzyme systems are reported.

5. Attention is called to the fact that glucosides, containing the phenols corresponding to the respective phenolases, are found in the plants examined.

6. The oxidized forms of these phenols could be reduced in each case by means of ascorbic acid, thus showing that the systems are reversible under natural conditions.

The writer wishes to express his indebtedness to Dr. J. P. BENNETT for the valuable aid and suggestive criticism offered throughout this work, to Dr. W. V. CRUESS for his help and the permission to use the facilities of his division, and to Dr. D. M. GREENBERG for valuable suggestions.

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# EFFECT OF ULTRAVIOLET RADIATION ON INDOLE-3-N-PROPIONIC ACID

DOROTHY HARE AND H. KERSTEN

(WITH SEVEN FIGURES)

## Introduction

Many effects on living as well as on non-living materials have been produced by ultraviolet radiation so that, in view of the recent interest in the group of organic chemicals known as plant hormones, it seemed advisable to the writers to investigate the effect of ultraviolet radiation on one of such substances. Indole-3-n-propionic acid was chosen because it could be obtained easily and because its effect on plants could readily be determined. The work was divided into two parts: one concerned with the effects of the radiation on the acid, and the other with the effects of the irradiated acid on the roots of plants.

## Materials and methods

In all of the experiments described, the source of the ultraviolet light was a water-cooled Victor mercury arc operated at 50 volts. Twenty milligrams of the indole-3-n-propionic acid was dissolved in 1 liter of distilled water and irradiated in 500-cc. portions in a quartz cylinder having a Bakelite base attached to the cylinder with Picein. The center of the cylinder, which was 7 cm. in diameter, was placed at a distance of 15 cm. from the arc and the liquid was stirred mechanically during irradiation. Solutions irradiated in small quartz test tubes produced the same effect on plants as those irradiated in the cylinder. Since the change in the acid was not caused by the Picein or the Bakelite the larger container was used throughout.

## Experimentation

### EFFECT OF ULTRAVIOLET RADIATION ON INDOLE-3-N-PROPIONIC ACID

The following results were obtained from ultraviolet radiation of indole-3-n-propionic acid solutions.

a. Aqueous and alcoholic solutions of the acid are colorless. If they are irradiated for several hours they become yellow. Solutions exposed to the air for the same length of time and otherwise treated in a like manner except for the irradiation remain colorless.

b. Aqueous solutions of the acid are odorless. If they are irradiated for a short period they acquire an unpleasant odor which, after a longer period of irradiation, becomes faintly but distinctly fruitlike. This fruitlike odor

still can be detected in solutions which have been diluted to ten times their original volume.

c. Aqueous solutions of the acid evaporated to dryness at room temperature in a draft of air leave a slightly yellowish residue. Irradiated solutions evaporated to dryness in a similar manner leave a dark brown residue having an unpleasant odor not possessed by the residue from the unirradiated sample.

Alcoholic solutions of the acid evaporated to dryness at room temperature in a draft of air leave a residue of the same color as the acid which was originally dissolved. Solutions of the acid in alcohol, irradiated for eight hours in a quartz test-tube and evaporated to dryness in a similar way, leave a dark brown residue having a disagreeable odor which suggests the presence of indole.

d. Aqueous solutions of the acid reveal a bright blue fluorescence when irradiated with ultraviolet light. The intensity of this blue fluorescence gradually decreases during the irradiation. Its decay can be demonstrated photographically by the apparatus shown in figure 1, in which the light

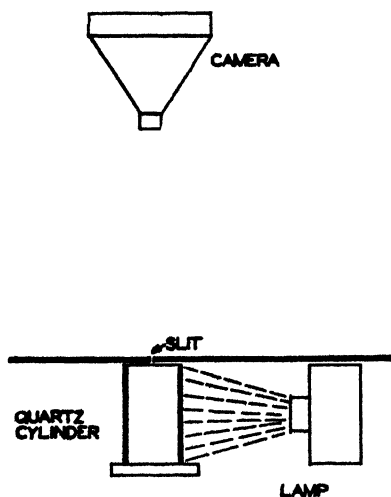


FIG. 1. Diagram of apparatus used to photograph decrease in fluorescence.

coming through a slit placed in the cover of the quartz cylinder is photographed at intervals during the irradiation. In this experiment the procedure was first to photograph the light coming through the slit when the quartz cylinder contained distilled water and then to photograph it at intervals during the time the solution of the acid was being irradiated in the cylinder. Between each photograph the film was shifted slightly so that the images of the slit appeared in adjacent positions on the film. During the intervals between photographs the cover containing the slit was removed

and the solution stirred with an electric stirrer. The result of this experiment was that a progressive decrease in the blackening was observed. This decrease was shown clearly when the relative blackening was measured by a Zeiss microphotometer (fig. 2).

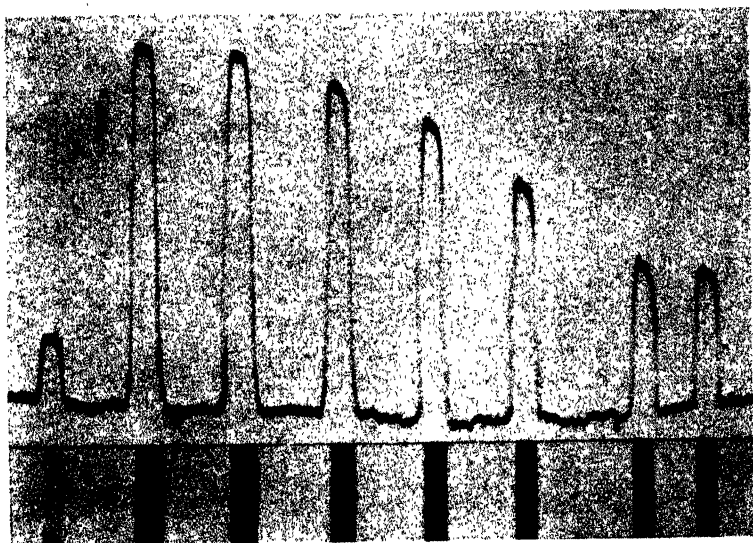


FIG. 2. Below, photograph of the fluorescent light from indole-3-n-propionic acid taken during irradiation with ultraviolet light with the arrangement shown in figure 1. Left to right, water, unirradiated acid, 15 min., 30 min., 1 hour, 2 hours, 3 hours and 4 hours of irradiation. Above, microphotometer photograph, made from the original negative, showing that the fluorescent light gradually disappears with time of exposure.

If a glass which absorbed the visible light but transmitted ultraviolet light was placed between the lamp and the quartz cylinder, the fluorescence appeared as a deeper blue and, although after a period of irradiation the solution remained clear in visible light, it seemed to be milky when lighted by ultraviolet light alone.

e. The fluorescence of the acid solutions extends into the ultraviolet region. This can be demonstrated by replacing the camera shown in figure 1 with a quartz spectrograph. The original spectrum from the ultraviolet lamp appears on the photograph taken in this way, but superimposed upon this is a broad band extending from about 4500 Å to about 3100 Å which gradually disappears as the irradiation progresses. Photographs taken at intervals during irradiation, as well as one taken when the quartz cylinder contained distilled water, are shown in figure 3.

f. Solutions of the acid exhibit a strong absorption band in the ultraviolet region which disappears during irradiation with ultraviolet light. Spectro-

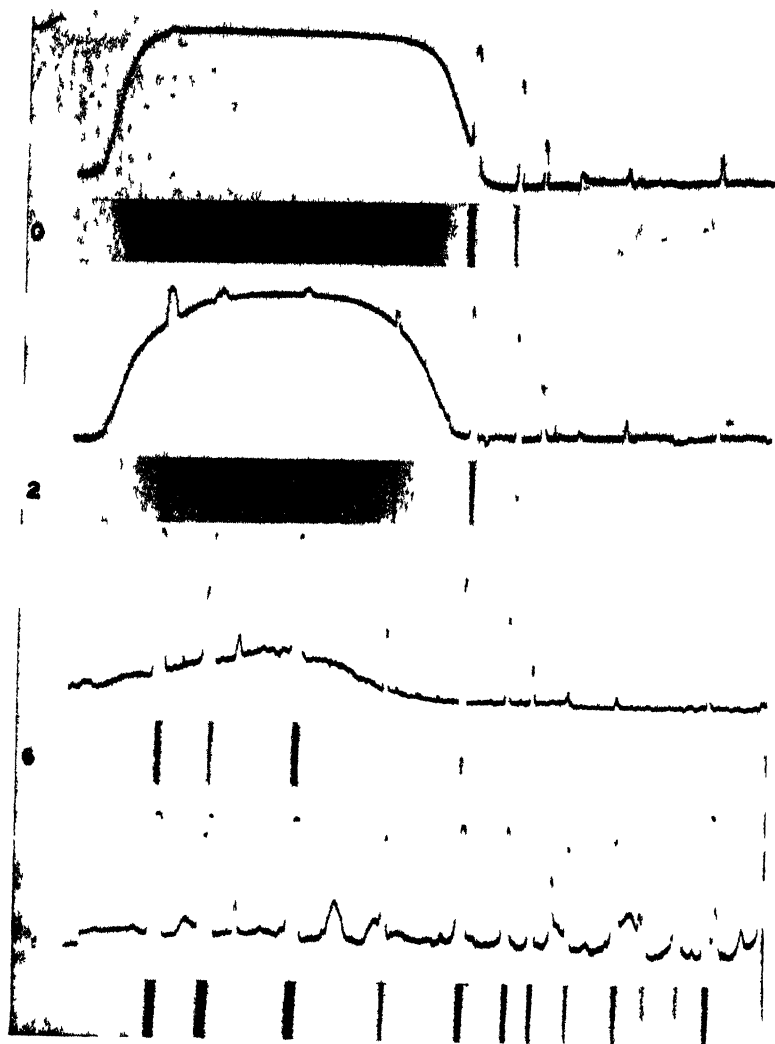


FIG 3. Spectrograms with the corresponding microphotometer photographs of the fluorescent light emitted by indole 3 n-propionic acid during irradiation with ultraviolet light. The lower spectrum is that obtained when the solution of the acid is replaced with distilled water. The numbers at the left of each set of photographs give the number of hours the solution had been irradiated before the photographs were taken

grams were taken with a Gaertner quartz spectrograph on Cramer contrast plates using a high frequency tungsten spark as the source of ultraviolet light. The solutions were contained in fused quartz precision cells during irradiation with the Victor mercury arc and while the spectra were being

photographed. The spectrograms, matched by the technique developed for this equipment by LOOFBOUROW (4), yield the curves shown in figure 4.

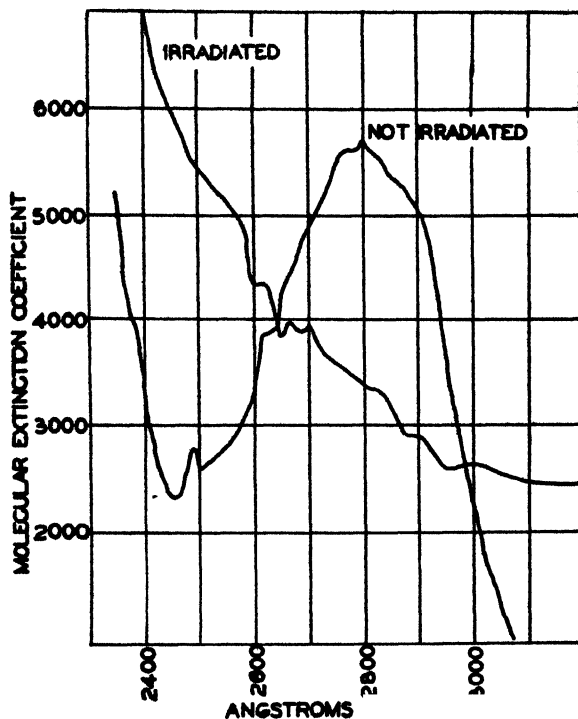


FIG. 4. Absorption curves of indole-3-n-propionic acid before and after 1 hour of irradiation.

#### EFFECT OF IRRADIATED INDOLE-3-N-PROPIONIC ACID ON PLANT ROOTS

It has been shown by HITCHCOCK (1) that the rooting of tomato and marigold cuttings can be retarded by proper concentrations of indole-3-n-propionic acid in water, and ZIMMERMAN and HITCHCOCK (7) have found that the aerial roots of tropical grapes were thickened abnormally and a large number of new roots developed when the root ends were immersed in growth solutions. Similar effects were observed by the writers when corn (Cross Golden Bantam = Purdue no. 51) and beans (Burpee's Stringless Green-pod bush bean) were grown in Knop's solution containing unirradiated acid. The effect was either absent or very much less in evidence when the acid used had been irradiated with ultraviolet light for several hours.

The seeds for this experiment were sprouted in clean, moist quartz sand. When the roots were from one-half to one inch long the seedlings were placed on cheesecloth stretched over a pan so that only the roots, projecting through the cloth, were immersed in water placed in the pan. After two



days of growth in this way they were transferred to pans containing the nutrient solutions.

A quantity of concentrated Knop's solution was prepared. This was divided into three equal portions. One portion was diluted with water so that the concentration of the salts in it were :

$\text{Ca}(\text{NO}_3)_2$ .....	1.00 gm. per liter
$\text{KNO}_3$ .....	0.25 gm. " "
$\text{KH}_2\text{PO}_4$ .....	0.25 gm. " "
$\text{MgSO}_4$ .....	0.25 gm. " "
$\text{FePO}_4$ .....	trace

A second portion was diluted with an unirradiated solution of the acid so that the concentration of each of the salts was the same as that of the first portion but the solution contained 2 mg. of indole-3-n-propionic acid per liter in addition to these. A third portion was similarly diluted except that an irradiated solution of the acid was used. Each of these three solutions was then placed in a flat enameled pan holding four and one-half liters of solution. Each of the pans was provided with a wooden cover containing fourteen one-inch holes. Young bean seedlings were supported in these holes by cheesecloth spread across the holes in such a manner that the roots dipped into the solution. The roots were compared after the plants had been grown in a greenhouse for from five to seven days. The results are shown in figure 5.

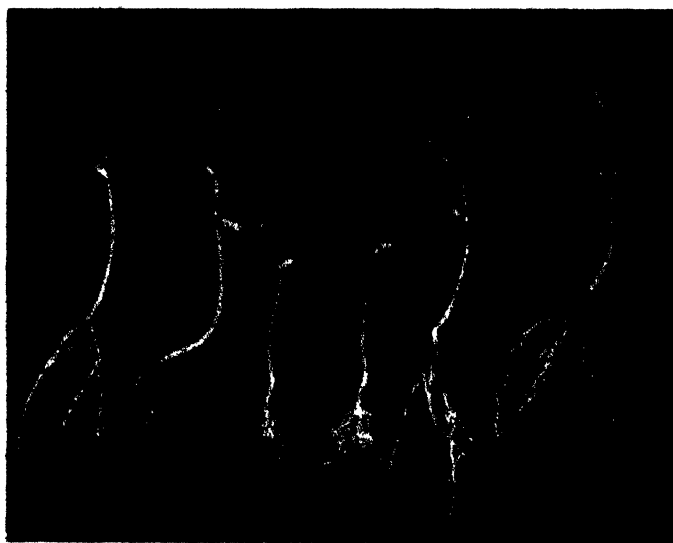


FIG. 5. Effect of indole-3-n-propionic acid upon the roots of bean plants. Left to right, irradiated acid, unirradiated acid, and control.

Roots of the plants placed in Knop's solution containing the non-irradiated acid solutions became thick and stubby and formed many branches. Frequently, the epidermal layer of the thickest roots split revealing scores of newly formed branching roots. The stems and leaves of the plants were usually stunted. On the other hand, roots of plants placed in Knop's solution containing the irradiated acid solutions grew in a normal or nearly normal manner. There was either no evidence or very little evidence of stunting in the stems or leaves of these plants.

Corn seedlings also produced abnormally thick and stubby roots in the unirradiated acid solution, and the leaves showed even greater stunting than that shown by the bean plants. The roots of seedlings grown in nutrient solutions to which a solution of irradiated indole-3-n-propionic acid had been added appeared normal (fig. 6).



FIG. 6. Effect of indole-3-n-propionic acid upon the roots of corn plants. Upper left, irradiated acid; upper right, control; lower, unirradiated acid.

The amount of non-irradiated indole-3-n-propionic acid used in the solutions was much greater than that required for the formation of abnormally thick, stubby roots. This was demonstrated in an experiment in which the non-irradiated acid was added to the solution in several different concentrations; namely, 2, 1, 0.5, and 0.25 mg. per liter, respectively. There was little

or no difference in the appearance of the roots of bean plants grown in the solutions containing 2 mg. and 1 mg. of the acid per liter. The roots of the plants in solutions containing 0.5 mg. of the acid were thick but not as stubby as those receiving more acid, and the roots in the solution with only 0.25 mg. of the acid appeared normal in size but showed an abnormal amount of branching.

Bean seedlings grown in nutrient solutions to which had been added solutions of indole-3-n-propionic acid irradiated for periods of 2, 4, 6, and 8 hours showed that there seemed to be a progressive destruction of the acid during irradiation. Figure 7, which shows plants grown in solutions of

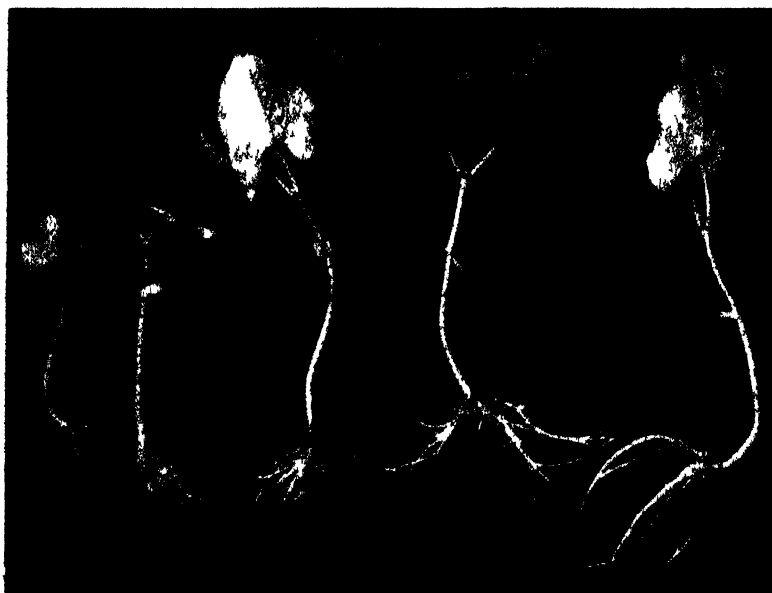


FIG. 7. Effect of indole-3-n-propionic acid solutions irradiated for various lengths of time upon roots of bean plants. Left to right, unirradiated, irradiated 2, 4, and 8 hours, control.

unirradiated acid, in acid solutions irradiated for 2, 4, and 8 hours, and in a nutrient solution, shows very clearly the gradual change which occurs in the acid in as far as it affects the development of normal roots. It was interesting to note that as long as there was any hint of the presence of a disagreeable odor the roots were not normal.

### Discussion

The evidence here presented points to the fact that a chemical change takes place in indole-3-n-propionic acid during irradiation of the solution

with ultraviolet light. This is not surprising since tryptophan which is  $\alpha$ -amino-indole-3-n-propionic acid has been shown to be destroyed by quartz lamp illumination (2). The destruction of both of these acids may be highly significant in plant life. We have shown that the destruction of indole-3-n-propionic acid is accompanied by a change in its action upon plant roots; therefore, a possible action of ultraviolet light upon the tryptophan within the plant may in some part explain the effect of ultraviolet light upon plants.

Since the authors were primarily interested in the physiological effect of indole-3-n-propionic acid, no particular effort was made to demonstrate just what change occurred in the material, although the changes in odor suggest that there may be first a breakdown to indole and then esterification of a product formed by a disruption of the indole ring. The odor of the product resulting after 8 hours of irradiation of the solution is strongly suggestive of that of methyl anthranilate which has a grape-like odor in dilute solutions and which has been shown to be present in grape juice (5). The idea that the indole ring in the acid is probably destroyed by ultraviolet radiation has further support (a) in that this breaking up by quartz-mercury lamp illumination of rings containing nitrogen in the nucleus has been demonstrated by LIEBEN and GETREUER (3) in the case of uric acid, xanthine, uracil, alloxan, hydantoin and  $\alpha$ -hydroxynicotinic acid, and (b) in the appearance of the absorption spectra before and after irradiation of the solution. The absorption spectra of solutions of the unirradiated acid show absorption which is characteristic of indole derivatives (6). This absorption band is destroyed when the acid is irradiated, indicating that indole compounds have disappeared from the solution.

The difference in the appearance of the roots as the amount of acid in the solution was decreased was very marked. There was a tendency for the roots to grow longer with only the region near the tip becoming swollen. Short branching roots grew in this region. A comparison of the effect produced in the plants by solutions irradiated 8 hours and of the effect produced by various concentrations of the unirradiated acid shows that the 8-hour-irradiation period must produce a destruction of more than seven-eighths of the active groupings. The roots produced by plants grown in acid solutions irradiated 8 hours were like those produced by the control plants grown in Knop's solution except that, in some instances, they were not quite as long as those of the control plants. This slight inhibition of growth may be explained in that there was still a very small quantity of the original acid remaining in the solution, or in that the products formed during irradiation have a slight physiological effect upon the plants. Since there is not always a difference in root length of the control plants and plants in the irradiated acid solutions, the first suggestion seems to be the more logical.

This work with the unirradiated acid upon corn and bean plants extends the field of activity of indole-3-n-propionic acid to two new species and outlines a procedure which is easy to follow. The results are consistent whether the plants are grown individually in dark bottles or all in one pan.

### Summary

Irradiation of indole-3-n-propionic acid with ultraviolet light from a mercury arc changes many of its physical properties and also changes its effect on the roots of plants.

The writers wish to express their thanks to Dr. D. A. WELLS, Dr. J. H. HOSKINS, and Dr. F. F. HEYROTH for advice and assistance on this problem.

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# FACTORS AFFECTING COLD RESISTANCE IN PLANTS

STUART DUNN

## Introduction

In testing cabbage and other herbaceous plants for cold hardiness (2) a considerable amount of variation in the reaction of individual plants was found. A given group of ten or more similar plants would show freezing injury varying from total death through stages of intermediate injury to those unharmed, when subjected to average killing temperature.

It became of interest to determine if methods might be devised to produce plants of a more uniform nature and not showing so great a variation in hardiness within a group. Any factors, environmental or otherwise, that would aid in producing uniformity in that respect, would also help to increase the average group hardiness. In the present paper studies have been made on the effect of soil moisture, mineral nutrition, growth temperature, and selection.

TYSDAL (7) working with alfalfa and WORZELLA (12) with winter wheat found that the plants survived artificial freezing tests to a greater extent in dry soils than in wet soils. However, the plants were not grown continuously at these different soil moisture levels but the soil was merely allowed to become dry to a certain percentage or wetted to a high one shortly before freezing the plants. This was also true of the conditions under which alfalfa was tested by WEIMER (8), winter wheat by HILL and SALMON (4), and alfalfa by PELTIER and TYSDAL (5), where plants in the dry soil were injured more severely than those growing in wet soil. It seemed desirable to determine the effect of growing plants continuously from the seedling stage on at different soil moisture levels, not only for amount of survival but for uniformity of individual reaction.

The effect of mineral nutrition on hardiness, particularly with respect to the elements nitrogen, phosphorus, and potassium, has been extensively studied by WILHELM (9, 10). He investigated the effects on hardiness of growing various plants such as wheat, oats, barley, rye, tomatoes, and beans in sand cultures with high, normal, low, and minus amounts of these elements supplied in the nutrient solutions. In general high amounts of potassium gave greatest survival of freezing temperature, and low and normal nitrogen, and low and normal phosphorus the same. The plants receiving none of any one of the elements being studied were usually the poorest in survival. CRANE (1) has found that fertilization with nitrate of soda caused peach buds to be susceptible to winter injury.

Most of the work on effect of growth temperature on hardiness has been in the nature of hardening experiments for comparatively short periods of time when the plants were partly grown and in which rather extreme tem-

peratures were used either continuously or with alternations with higher temperatures. This was true of the work of TYDSAL (7) and of TUMANOV (6). The former found that low temperatures alternating with higher ones produced hardier plants, and the latter found an intermediate continuous temperature more effective than a higher or lower one, but none of these as good as outside winter conditions. In the experiments reported here, the effect was determined of growing plants from seed continuously at different average mean temperatures. The effect of this sort of conditions has scarcely been reported, except for general observations on effects of different climatic conditions.

The value of vegetative selection as a means of increasing hardiness has, up to the present, not been given any consideration.

### Experimentation

#### EFFECT OF SOIL MOISTURE

Cabbage plants were grown in soil in glazed pots, which for some of the experiments were of about one-half-gallon capacity, and for others one gallon. Air-dry soil was evenly tamped in them and the pots allowed to stand in water until the soil was saturated. On the basis of this weight of water as 100 per cent. saturation other percentages of 50, 60, 70, and 80 per cent. were figured, and, after drying down to these points, seeds were planted. Each pot was kept up to its assigned moisture content by frequent weighings and addition of water.

Several different series of plants were grown at each of the above constant moisture amounts. After reaching a good size, in 5 to 6 weeks, they were placed in a cold storage room at  $-5^{\circ}$  C. for 15 hours and observations made on survival.

The results of all these tests when summarized were found to be very contradictory and inconclusive and did not give any direct evidence that high or low soil moisture content influences hardiness. Thus the tabular data will be omitted. There were also encountered the usual variations in injury by plants within a group indicating that this method of growing plants does not produce similar behavior in hardiness among individuals treated alike.

#### EFFECT OF MINERAL NUTRITION

Cabbage plants were grown in sand in glazed 1-gallon pots, 3 plants per pot. Forty pots were used for the study of each nutrient and they were divided into two lots, one receiving a nutrient solution containing a low amount of the particular element being studied and the other a high amount of it. The composition of the different solutions used may be seen in table I. The solution contained a complete amount of the other necessary minerals in each case and the solutions were supplied to the plants every 48 hours,

TABLE I

TABLE OF DILUTIONS FOR NUTRIENT CULTURES  
 MILLILITERS OF  $\frac{1}{2}$  MOL. SOLUTIONS OF SALTS MADE UP TO 1 LITER. DILUTED 10 TIMES  
 AND APPLIED AT 200 ML. PER POT EVERY 48 HOURS

SALTS USED	NITROGEN		PHOSPHORUS		POTASSIUM	
	HIGH	LOW	HIGH	LOW	HIGH	LOW
	ml.	ml.	ml.	ml.	ml.	ml.
K H <sub>2</sub> PO <sub>4</sub> .....	80	80	75	25	75	25
Ca (NO <sub>3</sub> ) <sub>2</sub> .....	120	20	75	75	75	75
Mg SO <sub>4</sub> .....	80	80	75	75	75	75
Fe Cl <sub>3</sub> .....	5	5	5	5	5	5
Ca Cl <sub>2</sub> .....	.....	100	.....	.....	.....	.....
K Cl .....	.....	.....	.....	50	50	.....
Na H <sub>2</sub> PO <sub>4</sub> .....	.....	.....	.....	.....	.....	50

with a thorough washing of the sand with water every week to remove accumulated deleterious substances. Thus the results on the effect of the lack or presence of each of these elements studied was obtained from a total of 60 plants for each treatment. The plants in all cases grew vigorously and were of uniform appearance. After they had attained a good size, usually at the age of about 2 months, they were tested for hardiness by exposure to a temperature of  $-5^{\circ}$  C. for 24 hours. All plants were grown in a cool greenhouse at a temperature of  $10^{\circ}$  to  $20^{\circ}$  C.

The results appear in table II. The plants grown with high and low nitrogen showed striking differences in size and growth up to the time of chilling. Those with high nitrate were much larger and more vigorous, but the plants in each group were very uniform in appearance. Two separate series of tests were run on this element and in each case the percentage of those uninjured by the cold does not differ greatly in the two treatments. The percentage difference for one is exactly the reverse of the other. In all of these tests, as with previous work, individual variations were found.

Plants grown in the same manner as above with high and low potassium solutions did not show any visible differences in growth and size up to the time of chilling. The usual variations in individual reactions were found here also. The figures indicate a slight advantage in favor of the low potassium group, but the percentage difference would indicate no real significance.

Two different series of high and low phosphorus plants were grown and tested, the plants being of uniform, vigorous appearance. In the first series the usual individual differences were manifest, but in this case it was the high phosphorus group that showed the greater hardiness, more pronounced.



**TABLE II**  
**EFFECT OF MINERAL NUTRITION ON HARDINESS OF CABBAGE**

ELEMENTS IN NUTRIENT	PERCENTAGE OF SURVIVAL	
	HIGH	LOW
	%	%
Nitrogen	45	50
Nitrogen	60	54
Potassium	62	68
Phosphorus	45	35
Phosphorus	42	40
Fluorine	Fluorine added 35	No fluorine 42

than that apparent between the high and low series of the other two nutrients. The next year the other series was grown to confirm this difference if possible. Individual differences within the groups of similar treatment again appeared but the total difference between the high and low series was so slight as not to be significant. It is evident then that this element, like potassium and nitrogen, has but slight influence on hardness.

The effect of fluorine on hardness was tried because of the results reported by WILSON (11) on the effect of various halogen salts on some characteristics of tobacco plants. He found that fluorides in small amounts furnished to plants grown in soil increased the bound water content of the plant, which, according to some investigators, is associated with hardness.

Accordingly cabbage plants were grown in sand culture in a similar way as for the other nutrient experiments, all of the plants being supplied with a complete nutrient solution. In addition half of the pots were treated with sodium fluoride. Beginning when the plants were 9 weeks old every other day each pot was supplied with 0.5 gm. of sodium fluoride dissolved in 200 cc. of its nutrient solution. About 8 days later the treated plants showed some slight signs of injury, such as yellow edges of leaves and reddened veins. On the twelfth day of treatment, the plants being good sized, both lots were chilled at  $-5^{\circ}$  C. for 20 hours. The results as given in the table show a greater total hardness in the untreated plants, indicating that fluorine is of doubtful value in inducing hardness.

#### EFFECT OF GROWTH TEMPERATURE

Although the percentage of soil moisture has but slight effect on hardness of plants, growing them in a soil of constant moisture content is of great value in producing plants of uniform size and vigor. Therefore this

method was used in growing cabbage plants at a soil moisture content of 70 per cent. saturation in glazed 1-gallon pots and potato plants at one of 60 per cent. saturation in glazed 3-gallon pots. Sixty of the cabbage plants and 25 potato plants were grown in a warm greenhouse kept constantly between 15° and 30° C., and a like number in a cold greenhouse at 10° to 20° C. On the average there was 5° C. difference in temperature between the two houses at all times. The seed stock of the potatoes used in this experiment is of particular interest because of its previous treatment. Twenty-five tubers were progeny of plants that had been grown continuously in the warm house for three years and in this experiment were again grown in the warm house. The other twenty-five had a similar history and treatment in the cold house.

When the plants were of good size, at an age of 2 months in each case, they were chilled; the potato plants at -1.1° C. for 20 hours, and cabbage at -5° C. for 24 hours. The results on potato for 3 successive years and on cabbage for 2 years are summarized in table III. They indicate that a

TABLE III  
EFFECT OF GROWTH TEMPERATURE ON HARDINESS

PLANTS	NO. OF CROPS	PERCENTAGE OF SURVIVAL	
		PLANTS GROWN AT 10°-20° C	PLANTS GROWN AT 15°-30° C.
Potato	1	% 94	% 32
	2	59	38
	3	62	45
Cabbage	1	35	29
	2	70	56

colder growth temperature is effective in producing a group of plants more hardy than those grown at a warmer temperature. Nevertheless, here as in the case of plants grown under other variations of environmental conditions there was no uniformity of behavior of individuals in any one group of plants.

#### EFFECT OF SELECTION BY VEGETATIVE PROPAGATION

As has been emphasized hitherto, in any given lot of plants there is a marked tendency toward individual variation in hardiness from total killing to no injury. It has been further shown that constancy of environmental factors, such as soil moisture content and growth temperature, does not produce greater uniformity in that respect.

It seemed desirable then to determine if these individual variations could be isolated or passed on from one generation to the next; that is, whether or not a uniformly hardy strain of plants could be produced by selection. Because of genetical difficulties and other complications involved in growing plants from seed, vegetative propagation was used. For this work *Bryophyllum* and Jerusalem artichoke were used as they are easily propagated vegetatively, the former by leaf cuttings and the latter by tubers.

A large number of *Bryophyllum* plants were started by leaf cuttings in sand from ordinary greenhouse stock. The plants were grown in soil in pots until of fair size and then chilled at  $-1.1^{\circ}\text{C}$ . for 15 hours. This was found to be about right for the threshold killing temperature, as it was for potato plants. When first subjected to this temperature a large number of the plants were killed, but cuttings were made from the survivals and other plants of the second generation raised from them. When these had all attained sufficient size they were again chilled and the process repeated for several generations. The time required for each generation to be grown to a certain size varied from 4 to 6 months in most cases and thus they were spread out over all seasons of the year, but at a warm temperature in all cases. Along with them were chilled a considerable number of plants of ordinary stock started from cuttings simultaneously with those of the selected stock and grown to the same age, under the same conditions, for comparative purposes. The results of these tests for six generations are summarized in table IV.

TABLE IV

EFFECT OF SELECTION BY VEGETATIVE PROPAGATION ON HARDINESS OF *BRYOPHYLLUM*

No. OF GENERATIONS	STOCK SELECTED FROM HARDY INDIVIDUALS		UNSELECTED STOCK	
	No. OF PLANTS CHILLED	SURVIVAL	No. OF PLANTS CHILLED	SURVIVAL
1 .....	.....	%	40	%
2 .....	63	73	100	32
3 .....	102	77	121	50
4 .....	135	65	100	53
5 .....	221	54	129	36
6 .....	271	40	250	39

These results indicate that it is possible to build up temporarily a resistant strain by vegetative propagation, with the high point in this case coming in the third generation, but eventually the plants will revert back

toward the original condition. This is evident from the close agreement of the percentage of plants surviving in the two groups of plants in the sixth generation. Although the percentage of survival of the unselected stock in the third and fourth generations is rather high, the average of all the figures for this stock is 37, which again is very close to the final value of 40 per cent. survival of the selected plants.

The results of tests with Jerusalem artichoke were very similar to those on *Bryophyllum*, except that the reversion to the original status comes in fewer generations.

An explanation of the reason for this temporary rise in hardiness in a group of plants treated in this manner is not readily forthcoming. Comparison of these results to the time of year during which the generations were grown reveals nothing consistent as regards seasonal variations to which it may be due. Probably it is due to a stimulus or shock from the treatment which wears off after a time. Such reactions are not unknown among plants. HARVEY (3) suggests that hardening in cabbage is a cold shock response, in that subjecting plants to cold for a few hours daily is just as effective in hardening them as continuous cold and that a few days of this treatment is as effective, or more so, than many days. A sort of fatigue is also evidenced by the sensitive plant, *Mimosa*, after repeated contact or jarring stimuli, if the repetitions are close together.

It seems then, that selection of surviving plants and their vegetative propagation is not enough to hold hardiness at a high level. Probably in combination with some other factor of the environment, such as temperature of growth, the method might be more successful in producing a hardy strain.

### Summary

1. An outstanding characteristic of herbaceous plants, tested for hardiness by freezing, is the individual variation in reaction in any given group of plants. Experiments were tried to eliminate these variations and to increase the average resistance of a group of plants by varying some of the environmental conditions, such as soil moisture, mineral nutrients, growth temperature, and by means of vegetative propagation of survivals of lengthy exposure to cold.

2. Growing cabbage plants at different constant soil moisture levels did not eliminate variations of hardiness within a group, and did not increase the average hardiness of any group.

3. High and low amounts of the nutrients nitrogen, phosphorus, and potassium did not affect degree or uniformity of hardiness of cabbage.

4. A cool growth temperature for potato and cabbage did not eliminate individual variations within a group, but did cause a greater average survival from freezing than with those grown at a warm temperature.

5. Vegetative propagation of survivals of cold exposure in groups of *Bryophyllum* and Jerusalem artichoke plants for several generations indi-

cated that the average hardiness within such a group could be increased temporarily, but eventually fell back to the original condition. Probably the effect of some other factor acting in cooperation with selection, such as a cool growing temperature, is required for holding the average survival at a high level.

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# GAS CONTENT OF CRANBERRIES AND POSSIBLE RELATIONSHIP OF RESPIRATORY ACTIVITY TO KEEPING QUALITY<sup>1</sup>

WM. B. ESSELEN, JR. AND C. R. FELLERS

(WITH ONE FIGURE)

## Introduction

This investigation was carried on to determine the changes in the composition of the internal atmosphere and in the catalase activity of cranberries as affected by variety and temperature while the fruit is in storage.

Only a few papers have been published on the composition of the internal atmosphere of fruits. In much of the work that was carried on no attempt was made to correlate the results obtained with the keeping quality of the fruit. In this investigation the relationship between the experimentally determined keeping quality of cranberries and the composition of the internal atmosphere is noted. No previous published report on the composition of the internal atmosphere of the cranberry was found.

## Equipment for collecting and analyzing gas

Since the methods used by previous workers were not suitable for this particular investigation it was necessary to devise a method for collecting the gas contained in the voids of cranberries. The following method for collecting gas from small fruit is rapid and sufficiently accurate for this work.

The gas-collecting apparatus consists of a glass funnel inverted in a 1-liter beaker, containing approximately 500 cc. of water, freshly boiled, to expel any dissolved gases. The sample of cranberries, approximately 70 gm., is placed in the beaker of water and the inverted funnel is placed over the fruit. A length of rubber tubing connects the stem of the funnel with the gas-analysis apparatus.

For the actual analysis of the gas a modification of the original Orsat apparatus was used. This apparatus consisted of a gas-measuring burette and two bubbling absorption pipettes. The first pipette, used to absorb carbon dioxide, contains a 12 M solution of sodium hydroxide, while the second pipette used to absorb oxygen, contains an alkaline pyrogalllic acid solution.

The gas-collecting and analyzing apparatus is set up as shown in figure 1. By means of the water-level bottle the air in the funnel is removed, and the funnel and tube connecting the funnel with the Orsat apparatus are filled with "gas-free water" from the beaker. The water in the beaker is heated

<sup>1</sup> Contribution no. 253, Massachusetts Agricultural Experiment Station, Amherst, Massachusetts.



Fig. 1. Apparatus for collecting and analyzing gas.

until all of the gas is expelled from the voids and intercellular spaces of the cranberries. This gas collects in the upper part of the funnel. As soon as the gas is collected it is drawn into the gas-measuring burette by means of the water-level bottle. The gas is allowed to stand until it has come to the temperature of the apparatus, and then its volume is determined by holding the water-level bottle so that the water level is the same in the bottle as in the burette. The sample of gas is passed into the carbon dioxide absorption pipette, and then passed back into the measuring burette. The absorptions are continued until a constant reading is obtained in the measuring burette. The volume is read and the difference from the original volume represents the volume of carbon dioxide contained in the sample. After the volume of carbon dioxide is determined, the volume of oxygen is determined in a similar manner by bubbling the gas into the oxygen absorption pipette. The residual gas after the absorption of carbon dioxide and oxygen is calculated as nitrogen. The volume of each of the gases is expressed in terms of percentage of total volume of gas.

### Experimentation

#### EFFECT OF FREEZING AND SUBMERGENCE ON THE COMPOSITION OF INTERNAL ATMOSPHERE OF CRANBERRIES

In order to determine the effect of various environmental factors on the composition of the internal atmosphere of cranberries, several samples of

TABLE I  
EFFECT OF FREEZING AND SUBMERGENCE ON COMPOSITION OF INTERNAL ATMOSPHERE OF CRANBERRIES.  
SAMPLES FROM MILLIS, MASSACHUSETTS

SAMPLE	DATE HARVESTED	NO. OF DE- TERMINA- TIONS	OXYGEN	CARBON DIOXIDE	NITROGEN	CARBON DIOXIDE- OXYGEN RATIO (CO <sub>2</sub> /O <sub>2</sub> )	HISTORY OF SAMPLE
1	Sept. 4-10	3	% 16.06	% 7.52	% 76.41	0.468	Stored in ventilated barn for 25 days
2	Oct. 30	3	9.97	10.75	79.28	1.075	Dropped on bog when sample 1 was raked. When fruit was gathered it was floating in water, under $\frac{1}{4}$ inch of ice. Fruit in good condition
3	Oct. 25	7	13.24	10.57	76.19	1.252	Left on vines until time of picking. Under water 10 days. Fruit in good condition
4	Oct. 25	3	11.90	5.18	82.92	0.435	Left on vines until time of picking. Were all above the water on the bog and had been frosted. Fruit quite soft
5	Oct. 30	4	11.54	11.12	77.33	0.972	Left on vines until time of picking. Bog frozen over and fruit above the ice. About $\frac{1}{4}$ of sample was frosted and the other $\frac{3}{4}$ in apparently good condition
5	Oct. 30 Sound fruit Frosted fruit	2	12.48	5.02	82.47	0.404	



fruit were collected from a natural cranberry bog, at Millis, Massachusetts. The cranberries were of the Howes variety. Summaries of the composition of the internal gas and the carbon dioxide-oxygen ratio, as well as the environmental conditions to which each sample was subjected, are presented in table I.

Considering the carbon-dioxide ratio as indicative of the rate of respiration, the cranberries which were left on the vines had a much higher rate of respiration than those which had been harvested about a month earlier and placed in storage. Since a high rate of respiration is detrimental to fruit, it would seem advisable not to leave the cranberries on the vines too long after they are mature.

TABLE II

COMPOSITION OF INTERNAL ATMOSPHERE OF EARLY BLACKS STORED AT 3° C. (37.4° F.)

DAYS IN STORAGE	NO. OF DETERMINATIONS	OXYGEN	CARBON DIOXIDE	NITROGEN	CARBON DIOXIDE-OXYGEN RATIO (CO <sub>2</sub> /O <sub>2</sub> )	CONDITION OF FRUIT
		%	%	%		
0	10	12.73	6.12	81.04	0.48	Good
7	4	19.49	2.75	77.85	0.14	"
14	4	16.75	3.20	80.04	0.19	"
26	7	11.55	2.89	85.54	0.25	"
32	7	16.26	4.05	79.68	0.24	"
46	2	18.33	2.26	79.40	0.12	"
52	2	17.78	1.71	80.50	0.09	Fruit becoming soft and decayed
74	1	15.81	1.86	82.33	0.12	Fruit approximately 50% decayed
84	1	15.37	5.59	79.04	0.36	Fruit approximately 50% decayed

Of the cranberries which were raked early in September, the fruit which was left on the bog and submerged in water had a much higher rate of respiration a month after picking than those cranberries which had been in storage during this time.

Of the cranberries which were left on the vines, those which were submerged in water showed a higher rate of respiration than those which were above water and in the air. It is possible to correlate these results with those obtained by FRANKLIN (1), and also by WAKABAYASHI (9), who found that submergence weakened cranberries and made them more susceptible to disease and breakdown. A correlation of results indicates that the weakened condition of cranberries which have been submerged in water is due to the

increased rate of respiration. In cranberries which have been frosted the rate of respiration is materially reduced.

COMPOSITION OF INTERNAL ATMOSPHERE AS AFFECTED BY VARIETY,  
TEMPERATURE, AND PERIOD OF STORAGE

In order to determine the variations in the composition of the internal atmosphere of cranberries throughout a definite storage period, and at different temperatures, two varieties of cranberries, Early Blacks and Howes,

TABLE III

COMPOSITION OF INTERNAL ATMOSPHERE OF EARLY BLACKS STORED AT 24° C. (75.2° F.)

DAYS IN STORAGE	NO. OF DETERMINATIONS	OXYGEN	CARBON DIOXIDE	NITROGEN	CARBON DIOXIDE-OXYGEN RATIO (CO <sub>2</sub> /O <sub>2</sub> )	CONDITION OF FRUIT
		%	%	%		
0	10	12.73	6.12	81.04	0.48	Good
7	7	12.53	7.89	79.13	0.62	"
14	7	9.21	12.16	78.62	1.32	Fruit becoming soft, rot setting in
21	7	6.66	12.89	80.44	1.93	Fruit soft and shriveled
28	7	5.10	12.02	82.86	2.35	High percentage of fruit decayed and soft
35	7	10.13	10.47	79.39	1.03	Fruit very soft
40	3	11.64	7.79	80.56	0.67	Fruit very soft and shriveled
46	3	9.49	11.28	79.23	1.13	Fruit very soft and shriveled
53	3	9.27	9.32	81.40	1.00	Fruit very soft
61	2	10.33	7.80	81.87	0.75	
68	1	7.33	10.00	82.67	1.36	Fruit shriveled and dried
78	1	8.59	8.59	82.82	1.00	Fruit 100% soft, shriveled, rotten, and dried

were stored in a cold storage room at 3° C. and in a warm room at 24° C. Samples of the fruit were taken at weekly intervals and the internal gas was analyzed. In order to have representative samples, cranberries were taken from various parts of the storage container and mixed together to make a uniform sample. All of the cranberries used in this experimental work came from Wareham, Massachusetts.

The results of the weekly analyses of the samples of cranberries are given in tables II, III, IV, and V. Results are calculated as percentage by

volume of oxygen, carbon dioxide, and nitrogen, and the carbon dioxide-oxygen ratio is given.

### Discussion of results

The temperature of storage has a marked effect on the keeping quality of cranberries. The Early Blacks stored at 24° C. broke down and decayed in approximately twenty days, while those stored at 3° C. remained in good

TABLE IV

COMPOSITION OF INTERNAL ATMOSPHERE OF HOWES STORED AT 3° C. (37.4° F.)

DAYS IN STORAGE	NO. OF DETERMINATIONS	OXYGEN	CARBON DIOXIDE	NITROGEN	CARBON DIOXIDE-OXYGEN RATIO (CO <sub>2</sub> /O <sub>2</sub> )	CONDITION OF FRUIT
		%	%	%		
0	11	14.59	8.32	77.08	0.57	Good
7	4	13.94	8.80	77.25	0.63	"
14	1	15.66	7.83	76.51	0.49	"
24	3	12.42	7.98	79.71	0.64	"
27	4	15.92	6.53	77.64	0.41	"
37	6	13.65	9.32	77.02	0.68	"
44	3	13.49	8.80	77.70	0.65	"
50	3	15.57	7.24	77.18	0.46	"
58	3	13.47	5.28	81.22	0.39	"
76	2	10.40	6.82	82.77	0.65	"
81	3	15.95	5.75	78.29	0.36	Fruit slightly soft
87	3	15.30	6.08	78.61	0.39	" " "
109	2	14.94	6.63	78.42	0.44	Fruit slightly soft, otherwise in good condition
116	1	13.43	5.97	80.60	0.44	Fruit slightly soft, otherwise in good condition
151	2	14.19	5.92	79.88	0.41	Fruit slightly soft, otherwise in good condition
159	5	15.55	5.86	78.57	0.37	Fruit slightly soft, otherwise in good condition
168	3	15.45	5.27	79.25	0.34	Fruit in fairly good condition
197	3	15.49	4.79	79.71	0.31	Fruit 50 to 70% soft and unmarketable

condition for over three months. At a temperature of 24° C. the Howes also broke down in approximately twenty days; and at this warm temperature their keeping quality was no better than that of the Early Blacks. However, at a temperature of 3° C. the Howes remained in good condition for seven months.

Throughout all of the tests the internal atmosphere of the Howes had a much higher carbon dioxide content and a correspondingly lower oxygen

content than did the Early Blacks. Hence, the carbon dioxide-oxygen ratio of the Howes was considerably greater than that of the Early Blacks. These results indicate that the keeping quality of cranberries may vary with the carbon dioxide content and the carbon dioxide-oxygen ratio.

The nitrogen plus inert gas content of the internal gas of the cranberries is similar to that of the atmosphere, about 79 per cent., except when the fruit is respiring at a very rapid rate. When the respiration rate is high

TABLE V  
COMPOSITION OF INTERNAL ATMOSPHERE OF HOWES STORED AT 24° C. (75.2° F.)

DAYS IN STORAGE	NO. OF DETERMINATIONS	OXYGEN	CARBON DIOXIDE	NITROGEN	CARBON DIOXIDE-OXYGEN RATIO (CO <sub>2</sub> /O <sub>2</sub> )	CONDITION OF FRUIT
		%	%	%		
0	11	14.59	8.32	77.08	0.57	Good
6	9	7.21	13.66	79.21	1.89	Slightly soft
13	10	6.24	16.95	76.80	2.71	Fruit becoming soft
21	10	5.39	17.62	76.98	3.27	Fruit quite soft, rot setting in, unmarketable condition
28	7	5.14	18.49	76.21	3.59	Softness and rot increasing
37	7	4.64	17.24	78.11	3.71	Large percentage of fruit very soft
44	7	5.28	17.90	76.81	3.38	Most of fruit very soft
50	6	5.61	17.07	77.31	3.04	Most of fruit very soft
58	6	5.53	16.17	78.27	2.92	Fruit very soft
66	3	7.53	12.93	79.53	1.71	Fruit very soft
73	2	6.14	11.25	82.85	1.83	Fruit badly shriveled
80	1	5.73	11.46	82.81	2.00	Fruit in decayed condition

the increased carbon dioxide content apparently replaces some of the nitrogen. But in general it may be said that the percentage of nitrogen in the internal atmosphere of cranberries is quite stable. When the cranberries have stopped respiring, or the cells are dead, the composition of the internal atmosphere approximates that of the outside atmosphere, *i.e.*, 79 per cent. nitrogen and 21 per cent. oxygen.

When stored at a warm temperature, the Howes showed a very rapid increase in the carbon dioxide content and a correspondingly rapid decrease after the peak was reached. Under similar conditions the carbon dioxide

content of the Early Blacks showed a more gradual increase and decrease and the peak was lower than in the Howes variety. MAGNESS (5), KIDD and WEST (4), and HARLEY and FISHER (2) obtained similar results from their studies on the internal atmosphere of apples.

In the cranberry there is a definite amount of respirable material which must be used up during respiration, before internal breakdown sets in. If the fruit is stored at a high temperature this material is used up more rapidly, as is shown by the carbon dioxide-oxygen ratios. In a similar manner it is shown that when cranberries are stored at low temperatures this respirable material is used up very gradually with the result that the life of the cranberry is greatly prolonged.

It is interesting to note that the Early Blacks stored at 24° C. showed a secondary peak in the carbon dioxide content and in the carbon dioxide-oxygen ratio. This secondary peak occurred after the fruit was very soft and decomposed. The odor of the cranberries made it quite evident that fermentation was taking place within the fruit. In all probability this increase in the carbon dioxide content of the internal gas was due to the action of microorganisms on the decomposed fruit.

The data obtained from these tests indicate that by a study of the carbon dioxide-oxygen ratios, based on the composition of the internal atmosphere of cranberries, it is possible to determine the length of time that cranberries may be kept in cold storage before internal breakdown sets in, and the fruit is in an unmarketable condition. This information may be obtained by running a test on cranberries stored at a high temperature for approximately ten days in the fall, when the fruit is first placed in storage. By comparing the carbon dioxide-oxygen ratios it is possible to forecast ahead approximately how long cranberries may be kept in storage. Such a forecast of the keeping quality of cranberries should be of value to canneries and people using cold storage. The writers propose also to check this method against the "spore load" and incubation tests for keeping quality as described by STEVENS (8). The present studies supplement the reports on respiration in cranberries during storage by MORSE (6) and on the spoilage of cranberries after harvest by SHEAR, STEVENS, WILCOX, and RUDOLPH (7). In general, these results are in agreement with the findings of these investigators.

#### CATALASE ACTIVITY OF CRANBERRIES STORED AT 3° C. AND 24° C.

In conjunction with the study of the changes in the composition of the internal atmosphere of cranberries during storage the catalase activity of the cranberries was determined at weekly intervals. Two methods were used in the determination of catalase activity. The first method used was the potassium permanganate titration method, by which the hydrogen per-

oxide not decomposed by the catalase is determined by titrating with potassium permanganate. The amount of potassium permanganate solution used varied inversely with the catalase activity. This method was discontinued because the tannins and pigments present in the fruit also reduce the permanganate solution, and because the results obtained are not a true indication of the actual catalase activity.

The second method for catalase determination was very satisfactory. The principle of the method is the volumetric liberation of oxygen from hydrogen peroxide as described by HAWK and BERGEIM (3). While cranberries show a moderate catalase activity, there was no correlation between catalase and respiratory activity at 3° C. but there was a direct correlation at 24° C. In general, a high catalase activity is associated with poor keeping quality. There is a sharp increase in activity a short time before cranberries begin to show structural breakdown in cold storage. The volume of oxygen liberated from hydrogen in Howes or Early Black varieties stored at 3° C. varies from 2 to 4 cc., while at 24° C. this value is somewhat higher. The data were not sufficiently impressive to warrant further experimental work though four different lots of cranberries were examined at weekly intervals for several months.

### Summary

1. Submergence causes an increased rate of respiration in cranberries and an increase in the carbon dioxide content of the internal atmosphere. It is probable that the increased rate of respiration weakens the cranberry and makes it more susceptible to fungus disease, as has been observed by FRANKLIN (1) and also by WAKABAYASHI (9).

2. Frosting reduces the rate of respiration in cranberries.

3. There is an increase in the respiration of cranberries if they are left on the vines after the usual picking time or maturity.

4. The temperature of storage has a marked affect on the keeping quality of cranberries. Cranberries stored at 24° C. (75.2° F.) became soft and partially decayed within twenty days.

5. The carbon dioxide content and the carbon dioxide-oxygen ratio vary directly with the keeping quality of the cranberries.

6. The nitrogen content of the internal atmosphere of cranberries is relatively constant and approximates that of the atmosphere. The carbon dioxide and oxygen contents of the internal gas vary with the rate of respiration.

7. Cranberries contain a definite amount of respirable material which must be used up before cranberries show physical breakdown in storage. When there is a high rate of respiration these expendable energy reserves are used up more rapidly and the life of the cranberry in storage is correspondingly decreased.

8. By means of the carbon dioxide-oxygen ratio it is possible to forecast with fairly good accuracy several months in advance the keeping qualities of cranberries in cold storage.

9. There is no significant correlation between catalase activity and respiratory activity of cranberries in storage.

10. The potassium permanganate titration method for the determination of catalase activity in cranberries is inaccurate because of interfering substances which are present in the cranberry.

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# NATURE OF THE BLACKMAN REACTION IN PHOTOSYNTHESIS

ROBERT EMERSON AND LOWELL GREEN

(WITH THREE FIGURES)

## Introduction

Among those writing on the mechanism of photosynthesis, which include WARBURG (7), WILLSTÄTTER (9), and FRANCK (3), the opinion is widely held that the Blackman reaction, which limits the rate of assimilation at high light intensities and carbon dioxide concentrations, involves the decomposition of a peroxide, formed in a preceding photochemical reaction, by the enzyme catalase. The best experimental support of this theory is found in the work of WARBURG and UYESUGI (8), and YABUSOE (11), who compared the temperature coefficients and sensitivity to several inhibitors for the Blackman reaction and for the decomposition of added hydrogen peroxide. In these respects they found certain similarities between the two processes. Such differences as they found were readily explainable, they believed, on the ground that while the same enzyme, catalase, played a part in both cases, the substrate was probably different, being very likely some organic peroxide, in the case of the Blackman reaction, rather than hydrogen peroxide.

Largely as a result of this work, WARBURG (6) gave up his "acceptor theory" in which the Blackman reaction preceded the photochemical reaction, and in agreement with WILLSTÄTTER and STOLL (10) regarded the Blackman reaction as involving the decomposition of a peroxide previously formed in the photochemical reaction. The writers have made further comparisons between peroxide decomposition and the Blackman reaction, and believe WARBURG's conclusion should be reconsidered, especially in view of the prominent part played by peroxide in the recently proposed chemical mechanisms for photosynthesis. GAFFRON and WOHL (5), in a theoretical paper, have objected that the energy available for the assimilatory process is insufficient to permit the formation of hydrogen peroxide as an intermediate product; but this objection seems to us of minor importance in the present discussion, because no definite claim has been made that the peroxide formed must be *hydrogen* peroxide. WILLSTÄTTER and STOLL (9) are more inclined to the opinion that the action of catalase in photosynthesis is on some peroxidic compound other than hydrogen peroxide. GAFFRON and WOHL also believe the formation of some sort of peroxide is hardly to be avoided, and suggest, on purely theoretical grounds, a peroxide of organic nitrogen, still regarding catalase as responsible for the freeing of the oxygen produced in photosynthesis.



In support of YABUSOE's observation that the Blackman reaction and peroxide decomposition are both linear functions of temperature, GAFFRON and WOHL cite (incorrectly) FRENCH (4) as confirming this for the Blackman reaction. But FRENCH reported no experiments on assimilation. His measurements show that peroxide decomposition varies in accordance with the Arrhenius equation, and not linearly with temperature.

### Experimentation

The writers have compared the rates of the Blackman reaction and decomposition of hydrogen peroxide by the same organism used by WARBURG (*Chlorella pyrenoidosa*) and by a second species of lower assimilatory activity, *C. vulgaris*. Some confusion may arise as to the names of these organisms, because WARBURG has sometimes referred to his as *C. vulgaris*. The taxonomic differences between the two species are unsatisfactory and difficult to establish, but there can be no doubt as to their physiological differences, and the one used by WARBURG certainly corresponds with the one we call *C. pyrenoidosa*. In cultures of average density (1 mm.<sup>3</sup> cells per ml. of fluid) and at 25° C. it shows a maximum assimilation of about 25 times its own volume of carbon dioxide per hour. *C. vulgaris* grows much more slowly, and shows under similar conditions a rate of assimilation only ten to fifteen times its own volume per hour. We have cultivated both species in pure culture for over ten years, and have never failed to find these physiological differences, as well as many others of which no mention has been made in published papers because they have as yet no clear bearing on problems of photosynthesis. The two species were last determined by Fräulein CANNABAEUS, in the laboratory of Professor KOLKWITZ, Berliner Institut für Wasser-Hygiene, in 1927.

The Blackman reaction is the name given to the rate-determining process when photosynthesis is saturated with both light and carbon dioxide. We define its rate as the number of cubic millimeters of oxygen produced per hour by one cubic millimeter of cells under saturating conditions. This was measured manometrically in the usual way, using rectangular vessels of the type shown in figure 1. Such vessels were filled with a suspension of cells

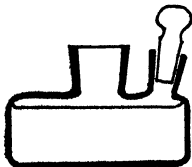


FIG. 1. Rectangular vessel used for determining rate of Blackman reaction.

sufficiently dense to give satisfactory readings on the manometer. The volume of cells used ran from about 2 mm.<sup>3</sup> for the more active suspensions

to 10 or 15 mm.<sup>3</sup> for the less active ones. As suspending fluid, either Knop's solution saturated with 5 per cent. carbon dioxide in air, or a carbonate mixture, was used. This mixture was prepared fresh for each experiment by mixing 85 parts M/10 potassium bicarbonate with 15 parts M/10 potassium carbonate. In either of these suspending fluids, large changes in carbon dioxide concentration resulted in only minor changes in the rate of assimilation. Saturating illumination, in which large changes in intensity produced no significant changes in rate of assimilation, was provided by a row of closely spaced 60-watt incandescent lamps whose tops were about 10 cm. from the bottoms of the vessels. A series of readings in the light was customarily followed by a dark reading, and the rate of respiration thus obtained was applied as a correction to the rate of photosynthesis. As a rule, the respiration correction is of no importance in establishing the rate of the Blackman reaction, because photosynthesis at light saturation is about twenty times as great as respiration. Nothing would be changed in our conclusions if the respiration correction were omitted altogether.

The rate of decomposition of hydrogen peroxide was measured in darkness, in vessels of the type shown in figure 2. Three ml. of cell suspension

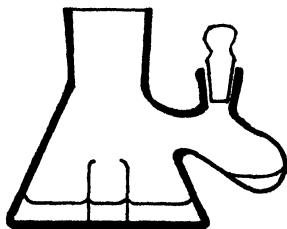


Fig. 2. Conical vessel used for determining rate of decomposition of hydrogen peroxide.

were filled into the main space, 0.3 ml. of 5 per cent. potassium hydroxide into the central well, and 0.3 ml. of M/30 hydrogen peroxide into the side bulb. Knop's solution was used as suspending fluid for the cells, and the peroxide solution was prepared by diluting Merck's "superoxol."

After attainment of temperature equilibrium, the peroxide was mixed with the cell suspension, and the oxygen production followed manometrically at 5-minute intervals, usually for about half an hour. The amount evolved in the first 15 minutes was used as a basis for computing the rate, which we have expressed in the same units as the Blackman reaction, to make the two rates readily comparable: mm.<sup>3</sup> of oxygen produced per hour per mm.<sup>3</sup> of cells. The experimental set-up and method of handling the data are essentially the same as were used by WARBURG and his collaborators, and, as we shall see, give much the same results. It is important to use a

minimum amount of cells in proportion to the amount of peroxide, to avoid large changes in peroxide concentration during the first 15 minutes. WARBURG's practice was to use 50 mm.<sup>3</sup> cells in 10 ml. fluid for each determination. The writers have found it practicable to use somewhat fewer cells, from 2 to 10 mm.<sup>3</sup> in 3 ml. of fluid, depending on their activity. In our experiments there is no indication of a decline in rate of peroxide decomposition during the first 15 minutes, except in the case of *C. vulgaris* at 20° C. and over. This was due to gradual injury of the cells by the peroxide rather than to any fall in peroxide concentration, as we were able to show by following the process for longer periods of time (3 hours) and at lower concentrations of peroxide. For *C. vulgaris* it would be more favorable to use M/600 instead of M/300 peroxide, especially at 20° C. and above. This concentration is less suitable for *C. pyrenoidosa*, and the writers thought it better to use a standard technique throughout, especially since the injury to *C. vulgaris* by peroxide at the lower temperatures is so slow that it is negligible in our experiments. In our tables, the rates for *C. vulgaris* above 20° C. are probably 10 to 20 per cent. lower than they should be, but this alters nothing in our conclusions, and tends only to minimize, rather than magnify, the differences on which they are based, because *C. vulgaris* always has a much higher rate of peroxide decomposition and a smaller Blackman reaction than *C. pyrenoidosa*.

We have followed WARBURG's practice of making no correction for respiration in the peroxide experiments, on the supposition that peroxide, rather than molecular oxygen, would be used by the cells as a source of oxygen for respiration under these conditions (WARBURG and UYESUGI, 8).

## Results

For cultures of equivalent density, *C. vulgaris* regularly shows a rate of the Blackman reaction about half as great as *C. pyrenoidosa*, but a rate of peroxide decomposition up to ten times as great. Yeast cells, though they cannot be said to have any Blackman reaction, show a rate of peroxide de-

TABLE I

RATES OF BLACKMAN REACTION AND PEROXIDE DECOMPOSITION AT 22° C. FOR *Chlorella vulgaris*, *C. pyrenoidosa*, AND YEAST (*Saccharomyces cerevisiae*). RATES EXPRESSED IN MM.<sup>3</sup> OXYGEN EVOLVED PER HOUR PER MM.<sup>3</sup> OF CELLS

	<i>C. VULGARIS</i>	<i>C. PYRENOIDOSA</i>	YEAST
Rate of Blackman reaction	16.4	26.7	
Rate of peroxide decomposition	38.3	4.1	68.8

composition about twice as great as *C. vulgaris*. Some figures are shown in table I. Exact comparison of the *C. pyrenoidosa* rates with WARBURG's is not possible, because UYESUGI and YABUSOE do not specify the exact amount of cells used for individual experiments, but an approximate comparison can be made from their general statements. For peroxide decomposition, they report from 20 to 40 mm.<sup>3</sup> oxygen evolved by 50 mm.<sup>3</sup> of cells in the first 15 minutes. In the units of table I, this means a rate of 6.4 to 12.8. For the Blackman reaction they report rates of 8 to 25, in the units of table I. The value of 8, taken from one of UYESUGI's tables, appears rather low to the writers, due perhaps to his use of very dense suspensions—too dense, in our opinion, for adequate light saturation. However, Mr. SARGENT's experiments, conducted in this laboratory, show that rates as low as 8 may be obtained with cells from old and very dense cultures of *C. pyrenoidosa*. It is to be noted that WARBURG's experiments were at 20° C. while the rates in table I are for 22° C., a difference of minor importance.

In table II are rates for the Blackman reaction and peroxide decomposition for *C. vulgaris* cells grown in glucose cultures with different amounts

TABLE II

COMPARISON OF RATES OF BLACKMAN REACTION AND PEROXIDE DECOMPOSITION FOR *C. vulgaris* CELLS CULTURED IN GLUCOSE WITH DIFFERENT AMOUNTS OF IRON. FIGURES ARE FOR 20° C., AND SHOW MM.<sup>3</sup> OXYGEN PRODUCED PER HOUR PER MM.<sup>3</sup> OF CELLS

	IRON ADDED TO CULTURE	NO IRON ADDED	IRON REMOVED BY AUTOCLAVING WITH CaCO <sub>3</sub>
Rate of Blackman reaction .....	5.7	3.5	< 1
Rate of peroxide decomposition .....	16.0	18.0	16.4

of iron, by the method of EMERSON (1). The diminished rate of the Blackman reaction in the iron-poor cells is not accompanied by a lower rate of peroxide decomposition.

It will be noted that these rates of peroxide decomposition are lower than for *C. vulgaris* in table I. This is due in part to the use of glucose in culturing the cells for the experiments in table II. Glucose in concentrations of about 1 per cent. inhibits the peroxide decomposition 15 to 35 per cent., though it has no direct effect on the Blackman reaction. The apparent effect of glucose on the rate of peroxide decomposition may perhaps be due to the greater consumption of oxygen by the glucose cells.

There is room for some disagreement as to the significance of the figures in table II, because culturing the cells with various amounts of iron results

in changing the chlorophyll content (1). Nevertheless, even cells of very low chlorophyll content show the usual characteristics of the Blackman reaction when saturated with light and carbon dioxide. The rate should then be limited by the Blackman reaction just as truly as in cells having a normal amount of chlorophyll. Yet we find an undiminished capacity to split peroxide, accompanied by a greatly reduced Blackman reaction. Neither here nor in table I is there any correlation between the rate of the Blackman reaction and the capacity to split hydrogen peroxide.

Figure 3 shows the effect of temperature on the Blackman reaction (solid curves) and peroxide decomposition (broken curves) for both species

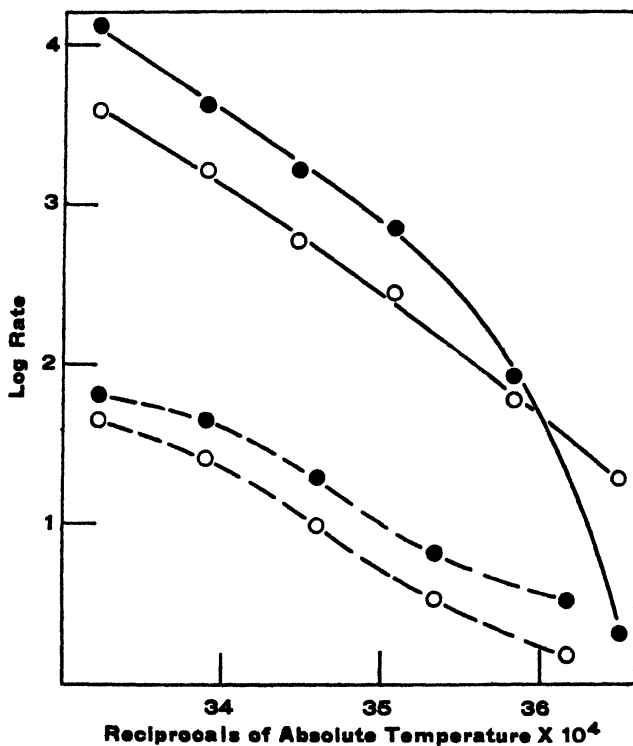


FIG. 3. Rates of the Blackman reaction and peroxide decomposition at different temperatures. Logarithms of rates are plotted against reciprocals of absolute temperatures. Solid curves are for the Blackman reaction; broken curves for peroxide decomposition. Solid circles refer to *Chlorella vulgaris*; open ones to *C. pyrenoidosa*.

of *Chlorella*. The solid circles are for *C. vulgaris*, the open circles for *C. pyrenoidosa*. The logarithms of rates are plotted against reciprocals of absolute temperatures, according to the ARRHENIUS equation. The positions of the curves on the ordinate axis are of no significance. Their slopes, which are independent of their positions, represent the temperature charac-

teristics ( $\mu$  in the Arrhenius equation). The rates and temperatures are given in table III, so that a direct plot can be made if desired. Such a plot

TABLE III

RATES OF BLACKMAN REACTION AND PEROXIDE DECOMPOSITION AT VARIOUS TEMPERATURES.  
DATA FOR FIGURE 3

TEMPERATURE	1/T <sub>ABS.</sub>	CHLORELLA VULGARIS		CHLORELLA PYRENOIDOSA	
		RATE	LOG. RATE	RATE	LOG. RATE
Blackman reaction					
°C.					
1	.003650	0.5	- 0.69	3.6	1.28
6	584	2.5	0.92	5.9	1.77
12	509	6.3	1.84	11.5	2.44
17	448	9.1	2.21	15.9	2.77
22	390	13.7	2.62	24.7	3.21
28	322	22.7	3.12	36.1	3.59
Peroxide decomposition					
3.5	.003617	12.4	2.52	1.2	0.18
10	534	16.8	2.82	1.7	0.53
16	460	26.8	3.29	2.7	0.99
22	390	38.3	3.65	4.1	1.41
28	322	45.2	3.81	5.2	1.65

shows the inadvisability of comparing temperature effects from experiments at only three widely separated temperatures, as did YABUSOE, for the Blackman reaction is not, as he supposed, a linear function of temperature, but has a shape fairly characteristic for other biological processes.

The curves for the Blackman reaction are reproduced here to facilitate comparison with those for peroxide decomposition, although they are almost the same as two published earlier in another connection (2, fig. 6). At that time the striking difference between the two species of *Chlorella* was pointed out. While their temperature characteristics are nearly alike for the upper range of temperatures (about 10,000), they differ widely at lower temperatures, for *C. vulgaris* running to about 50,000, and for *C. pyrenoidosa* only to about 20,000.

The curves for the peroxide decomposition leave little doubt that this process is controlled by the same mechanism in both species. The upper points for *C. vulgaris* tend to fall a little low, a manifestation of the injury from M/300 peroxide mentioned in the description of methods. It might be argued that the lesser downward curvature in the case of *C. pyrenoidosa* is due to the same effect, but there is nothing in the experimental readings to confirm this.

These curves for the effect of temperature on peroxide decomposition are very like FRENCH's (4) figure for *C. pyrenoidosa*. He draws a straight line through his points, instead of a curve. The writers' points seem to be somewhat better fitted by a curve. The differences, insignificant in any case, may be due in part to the fact that the writers' experiments cover a range of about 25°, while FRENCH's cover a range of 19°, and in part to his use of a method rather different from the writers'.

There is a remote resemblance between the curves for Blackman reaction and peroxide decomposition for *C. pyrenoidosa*, but surely insufficient to serve as evidence for a relationship between the two processes. Between the two curves for *C. vulgaris*, there seems to be no resemblance whatever.

### Conclusions

It is the opinion of the writers that an examination of the results presented in this paper suggests no significant similarity between the Blackman reaction and the peroxide decomposition. This leaves no experimental support for the hypothesis that the Blackman reaction involves the decomposition of some peroxide by catalase, but the writers wish to emphasize that neither these nor any other experiments of which they know constitute conclusive evidence that the Blackman reaction is *not* a peroxide decomposition. However, we suggest that WARBURG's "acceptor theory," abandoned in favor of the peroxide theory, is still worthy of consideration as a basis for constructing a mechanism of photosynthesis.

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# TUBERIZATION OF THE COLORADO WILD POTATO AS AFFECTED BY X-RADIATION

EDNA LOUISE JOHNSON

(WITH ONE FIGURE)

## Introduction

The Colorado wild potato (*Solanum jamesii*) is the only species among more than 100 different kinds of plants that the writer (6) has irradiated in which a plant organ has made greater growth leading to increased weight as a result of the treatment. The growth and development of the underground parts of 22 species (7) exposed to medium doses showed detrimental effects to adventitious, fibrous, and tap roots. Corms and bulbs of certain species of Iridaceae and Liliaceae, although less sensitive than tubers of the Jerusalem artichoke, agree in showing retarded growth. The wild potato was the only species, among those in which the underground organs especially were studied, for which a dose favoring tuber production was found.

JACOBSON (2) reported that by x-radiation the crop of one variety of cultivated potatoes was increased 84 per cent. in weight over the control plants, while with another variety the increase was as much as 200 per cent. He stated that not only was each tuber larger than normal but that there was an increase in the total number as well. SPRAGUE and LENZ (9) from preliminary experiments concluded that strong doses may reduce the number of tubers formed, but that such tubers attain a greater size so that the total production is not lowered.

In the writer's preliminary experiments (3) with cultivated potatoes grown in the greenhouse, tubers which were given light doses of x-rays formed more young tubers per hill than did the controls, but the average weight was less than that for the controls. Consequently the average total weight per hill was practically the same for controls and experimental plants. Since only a very limited number of cultivated potatoes could be grown in the greenhouse at one time, further experiments were carried on with the Colorado wild potato which has proved to be useful in ecological experiments (8) since it requires little room and matures quickly.

A summary (1) of the studies reporting stimulative action of x-radiation has pointed out that in the great majority of cases where it has occurred, light doses have been used. Experiments of two seasons, in which the use of light doses on the wild potato failed to give increased production, have been previously reported (5). Results based on 14,000 tubers obtained from the second year's experiment, indicated that the controls and experimental plants did not vary more in yield than would two groups of untreated plants.

In the study herein reported, the application of one dose of 1500 r-units to mother tubers was found to favor tuberization. Five different trials involving a large number of potatoes indicated that radiation of the unsprouted tubers resulted in only a slight increase in number of progeny and in average weight per hill, but treatment of sprouted tubers gave marked increase not only in average number and weight per hill but in average weight per tuber.

### Experimentation

#### UNSPROUTED TUBERS TREATED WITH MEDIUM X-RAY DOSAGE

Tubers were sorted so that those which were to serve as controls were of practically the same size and weight as the lot to be treated. Immediately after irradiation with a medium dose, the experimental tubers were planted either in plots in the University greenhouse, or outside in garden soil. In all cases, the controls alternated in both directions with treated tubers. When the crop was harvested about four months after planting, care was taken to sift the soil in order that even the very small tubers should not be lost.

TABLE I

PRODUCTION FROM UNSPROUTED TUBERS IRRADIATED WITH MEDIUM DOSES

	No. of HILLS	AV. NO. TUBERS PER HILL	DIFFER- ENCE IN TREATED PLANTS	AV. WT. PER HILL	DIFFER- ENCE IN TREATED PLANTS	AV. WT. PER TUBER	DIFFER- ENCE IN TREATED PLANTS
Tubers grown in greenhouse (Set-up for dose: 51 K.V. 7½ ma. 30 cm. 25 min.)							
Control.....	47	16.5	%	gm.	%	gm.	%
Irradiated...	57	17.0	+ 3.0*	9.5 11.1	+ 16.8	0.58 0.65	+ 12.1
Tubers grown in garden plot at altitude of 8000 ft. (Set-up for dose: 56 K.V. 5 ma. 30 cm. 33 min.)							
Control.....	50	19.5		13.8		0.71	
Irradiated...	51	20.3	+ 4.1	16.7	+ 21.0	0.82	+ 15.5
Tubers grown in garden plot at altitude of 5600 ft. (Dose: 1510 r-units)							
Control.....	48	20.3		22.6		1.1	
Irradiated...	43	20.8	+ 2.5	20.3	- 10.2	1.0	- 9.1
Totals from controls and 3 groups of unsprouted irradiated tubers							
Control.....	145	18.8		15.3		0.81	
Irradiated...	151	19.2	+ 2.1	15.6	+ 2.0	0.81	

\* In tables I and II of this paper, the plus sign indicates that the treated plants exhibited greater growth than the controls; the minus sign that the treated plants showed less growth.

In all cases the average number of tubers per hill, average weight per hill, and average weight per tuber were determined. These are recorded in table I. In one group which was grown in the greenhouse, it was found that the average length of time for sprouts of the irradiated tubers to appear aboveground was 36 days, whereas the average for the controls was 43 days. Dates of sprouting in the other groups were not recorded. In some cases experimental plants blossomed earlier than the check plants. Results from the three groups reported in table I indicate that when un-sprouted tubers are treated with medium doses of x-rays there is a slight increase in number of progeny and in average weight per hill.

#### SPROUTED TUBERS TREATED WITH 1500 R-UNITS

Tubers in the late spring were planted in soil in 3-inch paper pots. When the shoots had reached a height of from 1 to 2 cm., they were treated with a dose of 1500 r-units and placed in the ground outdoors without disturbance of the roots. Results obtained the first year indicated that this treatment was particularly favorable, for the number of tubers per hill was increased 59 per cent., the weight per hill 110 per cent., and the weight per tuber 30 per cent. The experiments, when repeated the following summer, confirmed the results of the previous year; 50 hills each of control and treated sprouted tubers yielded 79 and 113 tubers per hill respectively. The average weight of the progeny from the treated mother tubers was increased 50 per cent. and the experimental tubers averaged heavier than those from check plants (table II).

TABLE II  
PRODUCTION FROM SPROUTED TUBERS IRRADIATED WITH 1500 R-UNITS

	NO. OF HILLS	AV. NO. TUBERS PER HILL	DIFFER- ENCE IN TREATED PLANTS	AV. WT. PER HILL	DIFFER- ENCE IN TREATED PLANTS	AV. WT. PER TUBER	DIFFER- ENCE IN TREATED PLANTS
Tubers grown in garden plot at altitude of 5600 ft.							
Control	30	19.9	%	gm.	%	gm.	%
Irradiated	34	31.8	+ 59.6	17.5 36.8		0.88 1.15	
Tubers grown in garden plot at altitude of 5600 ft.							
Control	50	79.2		53.6		0.67	
Irradiated	50	113.4	+ 43.1	80.5	+ 50.2	0.71	+ 6.0
Totals showing production from controls and 2 groups of sprouted irradiated tubers							
Control	80	57.0		40.1		0.70	
Irradiated	84	80.4	+ 41.0	62.8	+ 56.6	0.78	+ 11.4

Figure 1 represents the entire crop produced the second year. The small size of the tubers can be appreciated when it is known that 3961 prog-

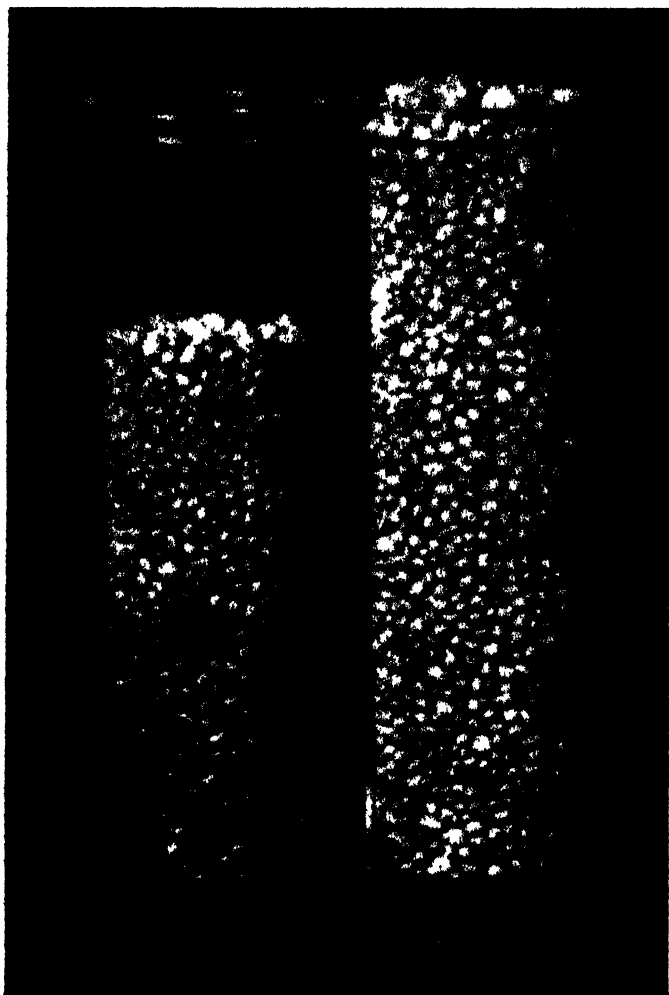


FIG. 1. Colorado wild potato tubers. Left, 3961 tubers from 50 control mothers; right, 5668 tubers from 50 sprouted mother tubers treated with 1500 r units. Average weight of tubers is approximately 0.7 grams.

eny from 50 control mother tubers are in the jar on the left and the 5668 offspring produced by the 50 irradiated sprouted mother tubers are on the right. The average weight of the tubers is about 0.7 grams.

The application of the correct dose of x-rays favors the production of wild potato tubers. Radiated unsprouted tubers produced a crop with a slight increase in number of tubers and also in average weight per hill. Treated sprouted tubers gave striking increases in number and weight per hill and in weight per tuber. The conclusion drawn is that the probable

increased rhizome development resulting in greater tuber production is similar to the increased aerial branching which occurs in some other members of this family (4) when very young plants are treated with medium doses.

### Summary

1. Radiation of wild potato tubers with light doses of x-rays failed to give increased number of progeny. Treatment of unsprouted tubers with moderate doses resulted in only a slight increase in number of progeny and in average weight per hill.

2. Progeny from sprouted mothers which had been treated with 1500 r-units gave the following percentage of increase over the controls: average number of tubers per hill, 41 per cent.; average weight per hill, 56 per cent.; average weight per tuber, 11 per cent. The explanation advanced for the greatly increased production from treated tubers is that there is a probable increased rhizome development which results in greater tuber production. This is similar to the increased aerial branching which occurs in some other members of Solanaceae when young plants are treated with medium doses.

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# CALCIUM AND BORON CONTENTS OF THE APPLE FRUIT AS RELATED TO THE INCIDENCE OF BLOTCHY CORK

W. A. DeLong

## Introduction

In the autumn of 1935 fruit and leaf samples of variety Stark apples were collected for the purpose of studying the contents of calcium and boron as related to the occurrence of the disorder known as blotchy cork, locally called "bitter-pit." These samples were obtained mainly from orchards which are under observation in connection with the investigation of the physiological disorders of the apple now in progress in the Annapolis Valley, Nova Scotia, fruit district. The method of collection followed was to obtain from each orchard two samples of fruit. One sample consisted of apples visibly affected with blotchy cork, the other of apples showing no visible symptoms of the disorder externally, and gathered from trees which apparently did not carry any affected fruit at the time the samples were collected. The samples were obtained on September 17 and 18, some weeks before the picking of this variety became general commercially. At the time that the fruit was picked two leaves also were taken from each spur from which apples were gathered. These leaves were all combined into two composite samples. One sample was from spurs bearing affected fruit, the other from spurs bearing apparently unaffected fruit on apparently unaffected trees. In connection with the sampling process it should be noted that the tree distribution of affected fruit was very irregular, as is frequently the case with this disorder, and that sampling distribution was correspondingly restricted. Thus, samples 7, 13, and 15 were collected mainly from the top sections of the trees from which they were picked, and more or less uniform distribution on the tree of affected fruit was found only in the case of samples 1 and 17. The general information concerning these samples is given in table I, in which the odd-numbered samples represent affected fruit (or leaves), and the even-numbered samples apparently unaffected fruit (or leaves).

## Experimentation

The fruit samples were prepared for analysis in the usual manner by paring and drying, analysis being made on the pulp only. The leaf samples were simply dried. Determinations of calcium were made according to the procedure recommended for the analysis of the ash of fruit products by the Association of Official Agricultural Chemists. For the boron determinations 40-gm. samples of the dried apple pulp were taken. To each sample was added an excess of 10 per cent. potassium carbonate solution, together with sufficient water thoroughly to moisten the apple tissue. The mixture was



TABLE I  
FRESH WEIGHT OF AFFECTED AND UNAFFECTED APPLES

SAMPLE NO.	TYPE OF ORCHARD SOIL	NO. OF FRUIT IN SAMPLE	AV. FRESH WEIGHT PER APPLE
			<i>gm.</i>
1	Sand, with sand subsoil .....	30	130
2	Sand, with sand subsoil .....	30	114
3	Loam, with clay subsoil .....	30	126
4	Loam, with clay subsoil .....	30	140
5	Loam, with clay subsoil .....	30	135
6	Loam, with clay subsoil .....	30	107
7	Sandy loam .....	30	163
8	Sandy loam .....	30	124
9	Sandy loam .....	30	116
10	Sandy loam .....	30	118
11	Sandy loam .....	28	115
12	Sandy loam .....	28	119
13	Sandy loam .....	30	108
14	Sandy loam .....	30	104
15	Sandy loam, with sand subsoil .....	30	117
16	Sandy loam, with sand subsoil .....	30	106
17	Clay loam underlain by sandy loam ..	30	161
18	Clay loam underlain by sandy loam ..	30	158
19	Composite leaf sample .....	.....	.....
20	Composite leaf sample .....	.....	.....
X	Sandy loam (fruit of 1935 crop) .....	38	100
	Sandy loam (fruit of 1934 crop) .....	40	120

then evaporated to dryness and ignited. The carbonaceous residue so obtained was made acid, lixiviated, again made alkaline with potassium carbonate solution, and the process repeated until ashing was complete. Ignition was at a temperature of about 450° C. The combined filtrates were made alkaline with potassium carbonate solution, evaporated to a small volume, transferred to 50-ml. volumetric flasks, and made up to the mark. Suitable aliquots were taken from the solutions so prepared for the colorimetric estimation of the boron content by the method of SMITH (4). This procedure has been found to be readily applicable to the determination of the amounts of boron found in apple tissue; the other ash elements present and the amounts of potassium chloride added during the ashing process exerted no measurable effect on the color change of the quinalizarin indicator. The method is convenient and rapid, and no difficulty has been experienced in obtaining duplicate determinations agreeing within the limits of accuracy of the procedure. Further, the results obtained were found, in the case of six solutions tested, namely, those from samples 1, 2, 3, 4, 5,

and 7, to be in agreement in respect to relative order of boron content with quantitative spectroscopic analyses for this element (3).

TABLE II  
ANALYSIS OF SAMPLES OF AFFECTED AND UNAFFECTED APPLES

SAMPLE NO.	PERCENTAGE ASH	CALCIUM IN P.P.M. OF FRESH WEIGHT	BORON IN P.P.M. OF DRY WEIGHT
	%	<i>p.p.m.</i>	<i>p.p.m.</i>
1 .....	0.234	36	11
2 .....	0.320	36	7
3 .....	0.239	32	16
4 .....	0.256	54	19
5 .....	0.289	36	22
6 .....	0.245	54	16
7 .....	0.329	33	11
8 .....	0.231	46	7
9 .....	0.261	32	11
10 .....	0.236	51	11
11 .....	0.252	28	19
12 .....	0.249	38	22
13 .....	0.293	30	7
14 .....	0.240	55	7
15 .....	0.261	36	16
16 .....	0.233	51	14
17 .....	0.296	28	11
18 .....	0.271	44	22
19 .....	.....	.....	27
20 .....	.....	.....	38
X .....	0.287	44	22 (1935 crop)
X .....	0.235	50	19 (1934 crop)

### Discussion

Examination of the results of the analysis (table II) fails to reveal any direct relation between the boron and calcium contents of the fruit, although, in view of the prevailing opinion that boron influences the calcium metabolism of the plant, some such relation might have been expected. Nor does it appear that there is any consistent relation of boron content to the incidence of blotchy cork. Yet, this too might have been expected to occur in view of the amelioration in the symptoms of other cork disorders, such as internal cork (corky core), obtained by boron treatment (1). In the case of the calcium content, however, the relation already reported (2), that a low proportion of calcium per unit of fresh weight of tissue is associated with the occurrence of blotchy cork, is again confirmed. This relation is quite marked in all cases examined in the present study except that of samples 1 and 2, in which the apparently unaffected fruits of sample 2 have essentially the same

calcium content as the affected fruits of sample 1. However, the recorded history of this orchard shows that practically all trees in it may be regarded as susceptible to blotchy cork. Storage results on fruit collected in 1935 confirm this opinion. Further, both samples 1 and 2 show a relatively low calcium content. It is therefore considered that this apparent exception to the rule, that fruits affected with blotchy cork are low in calcium, is actually more apparent than real.

### Summary

1. Parallel samples of variety Stark apples, some of which were visibly affected with blotchy cork and others which were apparently unaffected, were collected from a number of orchards and analyzed for calcium and boron content.

2. The results obtained do not support the hypothesis that blotchy cork is a result of boron deficiency, nor do they indicate that the boron and calcium contents of the fruit of the apple are closely related.

3. The results confirm the previous finding that blotchy cork is associated with a relatively low calcium content of the fruit.

The writer wishes to express his sincere appreciation for the cooperation of all those who have assisted in making this work possible. He extends thanks especially to Mr. C. A. HORTON of the Macdonald Physics Laboratory, McGill University, who made the spectroscopic boron determinations, and to Professors L. C. HARLOW and A. D. PICKETT, and their colleagues of the Nova Scotia Department of Agriculture who assisted in many ways.

MACDONALD COLLEGE  
MCGILL UNIVERSITY  
MONTREAL, CANADA

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## BRIEF PAPERS

### METHOD OF STIRRING GASES WITHIN A CLOSED CHAMBER

(WITH ONE FIGURE)

In various types of investigations it is often desirable to circulate gases within a tight chamber by a simple method which allows for ready adjustment of the rate of circulation. This is especially true in measuring respiration and carbon fixation by plants or respiration by animals. A suitable apparatus has been devised for this purpose. In principle the method consists of rotating a fan inside of a chamber by means of a magnetic coupling which operates through the glass wall of the chamber in such a way as to cause the inclosed fan to turn synchronously with a revolving electromagnet on the outside of the chamber.

A convenient arrangement of such a device is shown in figure 1. A

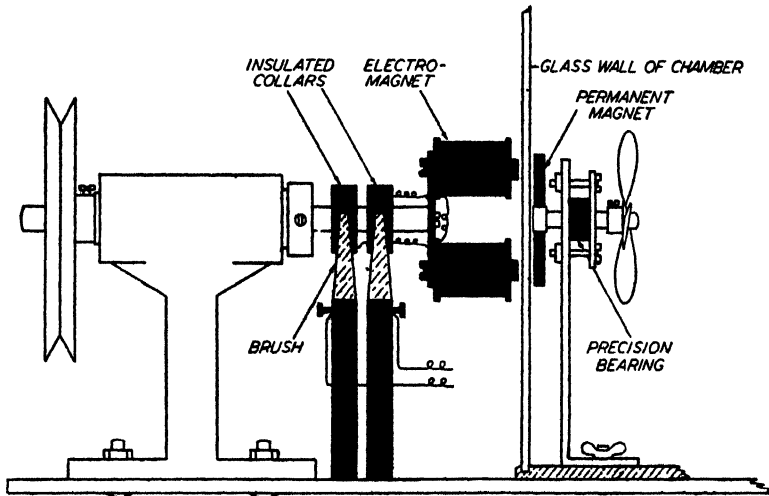


FIG. 1. Apparatus for stirring air within a closed chamber.

shaft, bearing an electromagnet and two insulated metal collars on one end, is supported by means of a suitable bearing. Copper brushes rest against the surface of the collars and carry electricity to the circuit of the electromagnet. A pulley is attached to the opposite end of the shaft and connected with a belt to a small motor by means of which the electromagnet is rotated. This part of the apparatus is mounted outside the chamber so that the ends of the core of the electromagnet are about one-sixteenth of an inch from the glass wall of the chamber and rotate in a plane parallel to it.

The removable fan unit that operates inside of the chamber consists of an L-shaped brass strip to which a precision bearing is attached by means

of a housing. The bearing supports a brass shaft, on one end of which is attached a permanent magnet, and on the other end, a fan. The unit is clamped in place by means of a bolt in the floor of the chamber so that the permanent magnet is about one-sixteenth of an inch distant from the wall of the chamber.

When the energized electromagnet rotates, the permanent magnet revolves synchronously with it, and in a plane parallel to the wall of the chamber. A suitable method of starting the apparatus is to cause the belt to slip and then gradually tighten until the maximum speed is reached as the two magnets remain synchronized only when subjected to a gradual rate of acceleration. Four units, equipped with four inch fans and electromagnets similar to those used in standard telegraph sounders, can be operated by current from an automobile generator. The amount of gas agitated by a given fan is varied by changing the speed at which the fan revolves and this is readily accomplished through the use of different sized pulleys.—JOHN W. MITCHELL and WILLIAM E. MARTIN, *University of Chicago*.

## NOTES

**Denver and Indianapolis Meetings.**—The American Society of Plant Physiologists has decided to meet with the A. A. A. S. at Denver in June, 1937, and at Indianapolis in December, 1937. Dr. H. F. CLEMENTS, Washington State College, is chairman of the program committee for the Denver meeting, and Prof. R. B. WITHROW, Purdue University, is chairman of the program committee for the Indianapolis meeting. The program committee has been authorized to determine the number of papers which may be accommodated at any given session, and to refuse papers which come in after the maximum number have been accepted for a given meeting. In addition, the committee may set time limits for all papers, and at future meetings each author is limited to one paper on the society's program. The restrictive measures are necessary, and will be administered in the interests of the entire membership. Sympathetic cooperation with the committees will solve any difficult situations that may arise.

The date of the Denver meeting has been fixed for the week of June 21–26, 1937. The programs of the plant physiologists will be held on June 22–25 of this week. A symposium on hormones is being arranged by Dr. F. W. WENT, and other symposia are contemplated. Inquiries regarding details of the meeting may be sent to the secretary, Dr. W. F. LOEHWING, or to the chairman of the program committee. General headquarters of the A. A. A. S. will be the Cosmopolitan Hotel. Other assignments have not yet been made.

**Western Section.**—The election held by the Western Section has resulted in the choice of Dr. J. P. BENNETT, University of California, as chairman; Dr. W. M. ATWOOD, Oregon State College, as vice-chairman; and Dr. H. F. CLEMENTS, State College of Washington, as secretary. The second annual meeting of the section coincides in time and place with the summer meeting of the society in Denver. It is hoped that many members residing outside of the regional territory covered by the Western Section may go to Denver, for it is an attractive meeting place, and the meeting offers opportunity for mid-western and far-western physiologists to become better acquainted. Fine fellowship, and the inspiration of scenic grandeur, will be enjoyed by all who come.

**New England Section.**—The annual meeting of the New England Section of the American Society of Plant Physiologists will be held under the auspices of the Rhode Island State College, at Kingston, Rhode Island, on May 14–15, 1937. Those who are planning to attend the meeting, or to take part in its discussions and deliberations are requested to inform Dr. B. E.

GILBERT, at Kingston, so that ample provision may be made for the comfort and convenience of all visiting scientists. A unique feature of the program will be sessions devoted to the problems of teaching plant physiology in different types of institutions.

**Frederick Frost Blackman.**—In celebration of the seventieth anniversary of his birth, this number of *PLANT PHYSIOLOGY* is dedicated by the American Society of Plant Physiologists to **FREDERICK FROST BLACKMAN**, who for many years was reader in plant physiology at the University of Cambridge. Dr. BLACKMAN was born in London, July 25, 1866. His parents were **FREDERICK BLACKMAN**, a doctor in South London, and **CATHERINE (FROST) BLACKMAN**, daughter of **WALTER FROST**, a doctor in Dorset. In 1917 he married **ELSIE CHICK**, daughter of **S. CHICK, J. P.** They have one child, a son.

Professor BLACKMAN's early education was obtained at the Mill Hill School, London, which he attended from 1878 to 1883. During several years immediately succeeding this period at the Mill Hill School, he prepared for the medical profession, and was an entrance scholar at St. Bartholomew's Hospital, London, in 1883. In 1886 he was awarded a gold medal by the University of London for his work in organic chemistry. Having become now deeply interested in the natural sciences, he gave up his medical studies in 1887, and entered St. John's College, Cambridge. Here he studied botany and plant physiology, and in 1891 he was appointed University Demonstrator in Botany. He was elected a fellow of St. John's College in 1895, and in the following year received the degree of D.Sc. (plant physiology) from London University. He was appointed University Lecturer in Botany (Musci and Algae) in 1897, and finally Reader in Botany at Cambridge in 1904, as successor to Sir **FRANCIS DARWIN**. This position he has filled with great distinction for 32 years. Many honors came to him in recognition of his leadership in his field. In 1906 he was elected Fellow of the Royal Society of London, and in 1908, president of the Botanical Section of the British Association for the Advancement of Science. From 1914 to 1917 he was a member of the council of the Royal Society of London, and in 1921 was awarded the Royal Medal of the Royal Society. Two years later he was Croonian Lecturer of this organization in recognition of his work on plant respiration. Honors also came from foreign countries as his fame spread to all nations. In 1926 he was elected a foreign member of the Academy of Science, Amsterdam, and in 1935 he was elected a **CHARLES REID BARNES** life member of the American Society of Plant Physiologists. In 1930 he served as president of the Physiology Section of the 5th International Botanical Congress which was held at Cambridge. His retirement as Emeritus Reader

in Plant Physiology, University of Cambridge, in 1936, permits leisure to continue his fruitful investigations on plant respiration.

It is appropriate to record a few of the many contributions which Professor BLACKMAN has made to science. Following his early work on organic chemistry with Dr. S. RUHEMANN, he began publishing in 1895 a series of experimental researches on vegetable assimilation and respiration. The first papers dealt with the paths of gaseous exchange in leaves, was followed by work on temperature and photosynthesis, optima and limiting factors, and a long list of papers contributing to our knowledge of photosynthesis and respiration. In 1928 he and his students started a series of papers which are essentially analytic studies in plant respiration. As emeritus reader, Professor BLACKMAN continues his work in this field.

Dr. BLACKMAN is versatile in his interests, and is fond of the fine arts, music, archeology, and is an amateur architect. He loves walking as a recreation, and is fond of travel. For many years he served as syndic of the Fitzwilliam Museum, Cambridge; was vicepresident of the University Musical Society; life member of the musical club; a member of the council of St. John's; a member of the Government Food Investigation Board; and director of the Cambridge Instrument Co. He is a life Fellow of St. John's College, member of the Athenaeum Club, and of the Wine and Food Society, London.

In dedicating this number of PLANT PHYSIOLOGY to Professor BLACKMAN, the American Society of Plant Physiologists recognizes the great value and significance of his contributions, and rejoices with him in celebration of the seventieth anniversary of his birth, which was reached on July 25, 1936. It is intended by this dedication to convey to Dr. BLACKMAN the hearty congratulations of American plant physiologists, and their cordial good wishes for a long and happy continuation of his distinguished service to humanity.

**James Bertram Overton.**—It is with keen regret that we must record the death, on March 18, 1937, of Dr. JAMES B. OVERTON, Professor of Plant Physiology at the University of Wisconsin. Dr. OVERTON was born at Richmond, Michigan, on December 23, 1869. He was educated in the public schools of Michigan, and received his Ph.B. degree from the University of Michigan in 1894. He began teaching at Black River Falls, Wisconsin, where he was assistant principal of the high school during 1894-95. He became senior master in mathematics at St. John's Military Academy at Delafield, Wisconsin, in 1895, a position which he held for several years. In the summer of 1900, and in 1901, he was assistant in botany at the University of Chicago, where he enjoyed association with Dr. J. M. COULTER, Dr. C. R. BARNES, and Dr. C. J. CHAMBERLAIN. He received the Ph.D. degree



from Chicago in 1901, his thesis subject being "*Parthenogenesis in *Thalictrum purpurascens*."*

For three years, following his work in Chicago, he was Professor of Biology at Illinois College, Jacksonville, Illinois, a connection which was honored in 1930 by the conferring upon him by that institution of the honorary Sc.D. In 1903-04 he became research assistant with the Carnegie Institution of Washington, and spent some time at the University of Bonn. He began his long service at the University of Wisconsin as instructor in botany in 1904. He was promoted to an assistant professorship in 1907, became associate professor of plant physiology in 1912, and professor in 1915. In this last position he gave almost 22 years of continuous service. During the years 1925-29 he renewed his connection with the Carnegie Institution as research associate. He held membership in many state and national scientific organizations, including the American Society of Plant Physiologists. At the Boston meeting of the plant physiologists, in 1933, Dr. OVERTON was honored by election as a CHARLES REID BARNES life member of the organization in recognition of his long service and his contributions to plant physiology. The immediate cause of death was heart failure. Mrs. OVERTON and several children survive him. His death is especially regretted as marking the first break in the living link memorial to Dr. BARNES, which was initiated at the Kansas City meeting in 1925, and at the Philadelphia meeting in 1926.

**Patrons.**—The American Society of Plant Physiologists provides in its constitution for four classes of membership: members, life members, patrons, and corresponding members. At the Atlantic City meeting, two members who some years ago had contributed liberally to the permanent support of the society were elected as its first patrons. These two members are Dr. WALTER THOMAS, Pennsylvania State College, and Dr. C. A. SHULL, University of Chicago. Naturally, these first patrons hope that they may not be left too long as the society's only patrons. There is plenty of opportunity for others to join the group, and the permanent funds of the society cannot be oversubscribed. This is a frank invitation to many others to share in the building of financial stability of the organization.

**Drawings.**—Occasionally the editors receive drawings which are inconveniently large for transportation by mail or express. The page width of PLANT PHYSIOLOGY is 4.5 inches, and drawings which must be made large enough to occupy the full width of the page should be drawn to a scale that calls only for one-half or one-third reduction, that is, nine to thirteen inches in width. Extra charges are encountered when excessive or unusual reduction in size must be made. Lines and figures should always be adapted in

thickness and size to the reductions contemplated. There seems to be no excuse, except lack of understanding, perhaps, for drawings made up to occupy several feet each way. In case the original drawings are large, it is suggested that properly reduced photographs be sent for reproduction. The author could then judge accurately whether the original lines and figures were heavy enough to show properly when reduced to the size which can be used in the journal.

**Vacation.**—The editor-in-chief of PLANT PHYSIOLOGY is spending the spring quarter of 1937 *in absentia* from the University of Chicago. It is suggested that those who have papers in preparation for the journal might find it convenient to hold them until about June 10, at which time the office will again be open. No attempts will be made to reply to first class mail during this period.

**Chromatographic Adsorption Method.**—About thirty years ago TSWETT published the first account of his chromatographic method of isolating carotenoid pigments from solutions. The usefulness of the method has been recognized, and extended, until now it is of major importance in the problems of pigment separation. A monograph devoted to the chromatographic technique, *Die chromatographische Adsorptionsmethode*, has been published by the Vienna press of Julius Springer. The authors are Dr. L. ZECHMEISTER and Dr. L. v. CHOLNOKY.

The work is presented in two main sections. The general section discusses the field in which the method is useful, its history, theoretical background, the relations between chromatographic behavior and constitutional formulae, and the methods applied to various pigments and colorless substances.

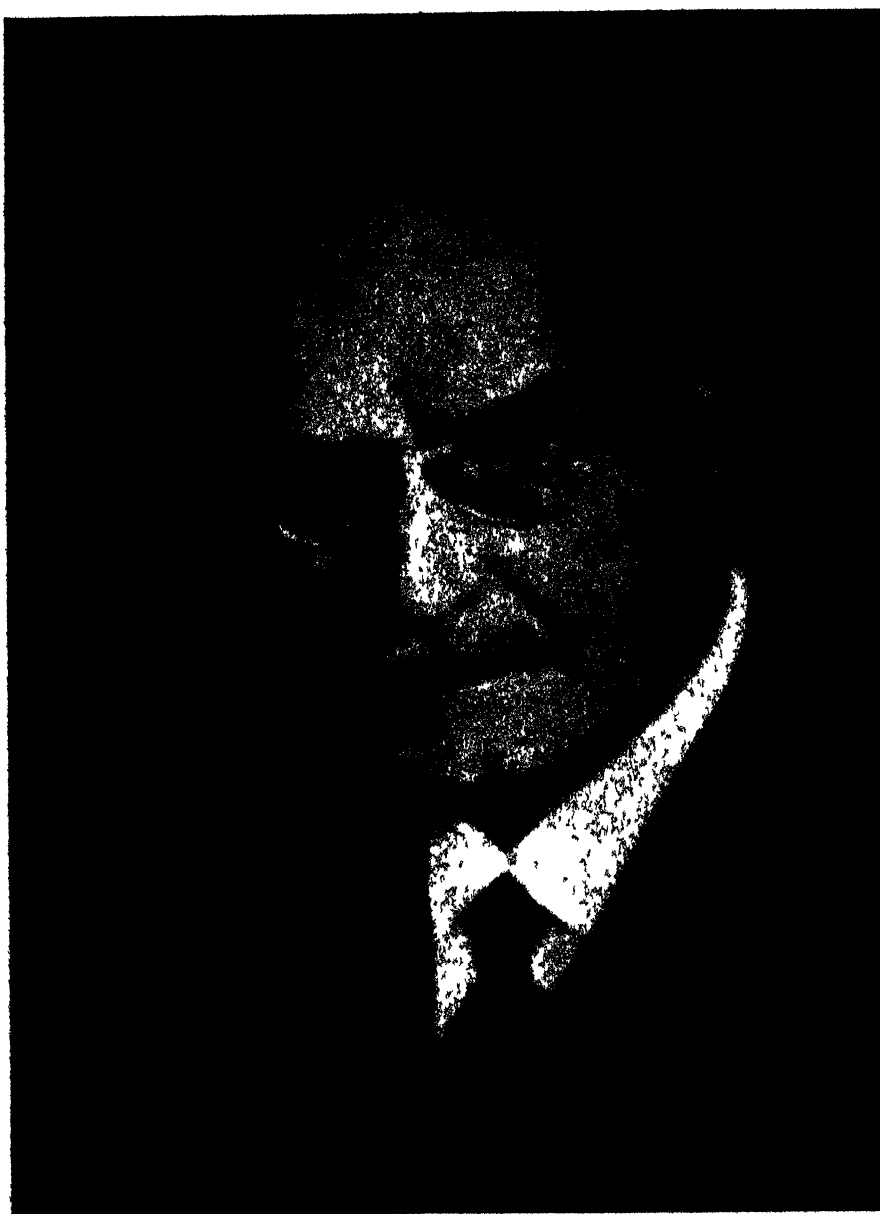
The special section discusses the application of the method to natural coloring matters, to artificial pigments, and to colorless and weakly colored substances. These special discussions will prove most helpful to those engaged in pigment study. The rapid extension of our knowledge of the carotenoids has been greatly favored by the development of efficient means of separation of closely related chemical compounds. The chromatographic method makes possible identification of the different pigments, and facilitates their quantitative estimation.

The book is published in brochure form, contains 191 pages of text, 45 figures, a bibliography containing several hundred citations, author and subject indices; the total number of pages is 231. The price of this excellent monograph is 14.40 RM per copy. Address orders to Julius Springer, I Schottengasse 4, Wien.

**Contractile Cells of Plants.**—Volume X of the *Protoplasma Monographien*, Gebrüder Borntraeger, Berlin, is entitled *Die kontraktile Zelle der Pflanzen*. The author is SILVIA COLLA, Privatdozent in Botany, University of Turin. Following a brief introduction there are seven chapters, dealing with material and methods, morphology of contractile cells, mechanism of movement of various contractile organs, the various phases of contractile phenomena, the laws governing the contraction and movements of isolated cells, physico-chemical changes during contraction, and a comparison of plant contractions with other cellular contractions, such as striped muscle tissue, contractile cells and organs among the protozoa, and other movements in plants. There is a long literature list (333 titles), and brief author and subject indices. There are 77 illustrations and 168 pages.

The monograph brings together much interesting and valuable information concerning the power of protoplasm to contract, and to move various organs. Especially valuable are the summaries of what is known of the physico-chemical changes occurring in cells during contraction, such as changes in potential, pH, rH, concentration of crystalloids, colloidal changes, and modifications of permeability. This volume should receive a hearty welcome from physiologists and biologists generally, because of its elucidation of the problems of protoplasmic behavior. The price, in the usual red cloth binding, is RM 12. Orders for the book should be addressed to the publishers, Gebrüder Borntraeger, Berlin W 35, Koester Ufer 17, Germany.





*Hans Molisch.*

JULY 12, 1856

# PLANT PHYSIOLOGY

JULY, 1937

## FIFTY YEARS OF PLANT PHYSIOLOGY IN AUSTRIA<sup>1</sup>

L. LINSBAUER

Plant physiology in Austria took a powerful upturn after the year 1873, the year in which JULIUS WIESNER brought into existence the first (not only in Austria, but anywhere) plant physiological Institut. It was the period in which this science under the three great stars, WIESNER, SACHS, and PFEFFER made great and decisive strides forward. It is understandable that very soon a host of enthusiastic young men gathered around the great teacher; and so it is no wonder that there went forth from this school many men who in subsequent years occupied professional chairs in the Austrian universities. Among the plant physiologists are mentioned the following: A. BURGERSTEIN (Wien), J. CZAPEK (Prag), W. FIGDOR (Wien), V. GRAFE (Wien), G. HABERLANDT (Berlin), K. LINSBAUER (Graz), and H. MOLISCH (Wien).

The situation was changed by the World War. The Austria of today, as those from foreign lands know it, has now only the universities of Vienna, Graz, and Innsbruck, which will be considered in this paper.

It is characteristic of JULIUS WIESNER that he was no specialist; the field of research in which he worked was many sided. Notwithstanding, there were a few main problems which occupied him through many years: Chlorophyll formation; the relation of the plant to light, which led to his photometric investigations and to the development of his concept of *Lichtgenuss*, which was of importance in ecological research; the causes of orientation of plant organs (anisomorphy, exotrophy, anisophylly), to mention only a few.

WIESNER liked to put his experiences to the service of practical needs. Best known is his great work "*Die Rohstoffe des Pflanzenreiches*"; less well known is the essential service which he rendered to the sugar industry when he introduced the dialysis procedure which has been used ever since.

There is a peculiar charm to be seen in the fact that WIESNER also had an interest in the more general scientific problems, and gave expression to his thoughts concerning them. In one of his most important and funda-

<sup>1</sup> Translated from the original German by Dr. Frank M. Andrews, Indiana University.

mental works, *Die Elementarstruktur und das Wachstum der lebenden Substanz*, he put forward the theory that the cells are not the ultimate units of living material, but are made up of a sum of plasomes which already possess all of the properties of life. At the same time he viewed the cell not as a mere aggregation of individual particles, and the life processes of the plant not as accidental occurrences; but he thought that the driving mechanism of the plant's life is regulated by an internal order which WIESNER called *Enharmonie*. Such trains of thought, which remind one of the modern conceptions of life as a fundamental unity, show a notable breadth of comprehension.

WIESNER visited the United States in a private capacity, so to speak, on way of a trip to the Yellowstone region where he undertook photometrical investigations. He also published a paper, *The development of plant physiology under the influence of the other sciences*. (Congress of Arts and Science. Universal Exposition, vol. V, pp. 103-124. St. Louis. 1904.)

HANS MOLISCH, the successor of WIESNER, occupied the entire last half century with his scientific investigations. It is utterly impossible in a limited space to do justice to the labors of this industrious Nestor among Austrian botanists. We shall therefore attempt to give only a very general summary of his works, the number of which runs to about 200. His plant physiological investigations (apart from the purely anatomical ones) have enriched almost every chapter of this science. Here there are mentioned only a few remarkable papers which, as independent publications of preliminary studies are united into a complete account: *Die Pflanze in ihren Beziehungen zum Eisen* (1891); *Untersuchungen über das Erfrieren der Pflanzen* (1897); *Studien über den Milchsafte und Schleimsafte der Pflanzen* (1901); *Leuchtende Pflanzen* (1904); *Die Purpurbakterien* (1907); *Die Eisenbakterien* (1910).

The microchemistry of plants has experienced extraordinary advancement through numerous individual investigations by MOLISCH, and his *Mikrochemie der Pflanzen* (1913) is indispensable to every plant physiologist and plant anatomist. It should be mentioned in this connection, that plant chemistry and microchemistry were cultivated with success in Austria; well known is the fundamental *Biochemie der Pflanzen* by CZAPEK. In addition V. GRAFE worked in Vienna, and G. KLEIN wrote his *Histochemie der Pflanzen*, while general microchemical methods had as their outstanding devotees, PREGL and EMICH, in Graz.

MOLISCH's *Grundriss einer Histochemie pflanzlicher Genussmittel* (1891) had already served decidedly practical ends, and so he, the son of an old horticultural family, had especially at heart the needs of horticulture. He served these needs in his valuable study, *Das Warmbad als Mittel zum Treiben der Pflanzen* (1909). His main effort in this direction, however,

produced the standard work *Pflanzen Physiologie als Theorie der Gärtnerei*, which, having appeared in 1916, has since reached its sixth edition.

For the purpose of diagnosis in various fields, the paper *Aschenbild und Pflanzenverwandtschaft* (1920) offers valuable materials. MOLISCH also wrote a *Physiologie der Pflanzen* (1917), an *Anatomie* (1920), a fascinating book, *Botanische Versuche ohne Apparate*, and in addition a series of others in which he shows himself to be a master at popular presentation. All of the works of MOLISCH are distinguished by great exactness of observation, so that they possess documentary value. His arrangement of experiments is often of classical simplicity. And often, from apparently insignificant observations, he obtained important new facts. Because of the unusual breadth of the research field treated by him, the possibility presents itself to extend his work at many points. "That, indeed, is the best that a scientific work can offer, to give living impulse to new investigations." (WIESNER).

MOLISCH made extensive foreign trips. His impressions and observations in India and Japan, where he was invited to the organization of a biological institute, were reported in three books of travel, 1926, 1927, 1930. On his return trip he briefly visited the United States where he became acquainted with the famous Boyce-Thompson Institute. MOLISCH, who has recently become 80 years old, is vice-president of the Academy of Science in Vienna, and was at the time of the celebration of his birthday honored and accorded distinction from many places.

After MOLISCH, followed G. KLEIN, who concerned himself chiefly with plant chemistry; but he remained in connection with the institute only a short time.

During the years 1930-1934 F. C. VON FABER was the director of the WIESNER Plant Physiological Institute. As a tropical botanist with rich experience, and as a pioneer of free land botany from Java, he organized in the vicinity of Vienna a small free land station. Work along his line will be continued in Vienna. Before his departure for Munich he had modernized the institute.

With K. HÖFLER another of MOLISCH's students assumed the teacher's chair. HÖFLER is first of all a representative of cell physiology, which he developed in connection with several young co-workers. Also other representatives of this flourishing Austrian work have gone forth from MOLISCH's school, as WEBER in Graz, and GICKELHORN in Prag.

HÖFLER first studied the osmotic values of the plant cell. By the use of his plasmometric method which permits one to express the degree of plasmolysis of the protoplasm numerically, he investigated the permeability of various substances, and the distribution of permeability in the different tissues of a plant. He was able to show, moreover, that it is possible to



characterize certain systematic units of the plant kingdom by definite physical properties of the plasma, in the same way as it is possible to do from morphological and anatomical characters, and has thus shown the way to a "comparative protoplasmatics."

Jointly with R. CHAMBERS (New York) he investigated the tonoplast of *Allium cepa* whose membranes are looked upon as extremely thin fluid films composed of materials not miscible with water.

To round out my account I will mention that as older physiologists of the Vienna school, A. BURGERSTEIN (transpiration monograph) and W. FIGDOR (monograph on anisophylly) were still active. Moreover, investigations in the field of plant physiology were carried on in the technical agricultural school in Vienna, where J. BÖHM especially had occupied himself with the problem of sap ascent. Furthermore, from the Biological Experiment Station there were produced physiological papers under the leadership of FIGDOR and L. VON PORTHEIM and their students.

To these is added here O. RICHTER, likewise one of MOLISCH's students. At an early time in the now foreign University of Brünn he has for a long time made himself known by his physiological investigations of nutrition. He concerned himself with the nutrition of the algae, grew the Diatomaceae in pure culture, found that they can assimilate organic substances and that magnesium is indispensable for them, studied the relation of magnesium to the plant, and investigated the nutritional physiology of cultivated grasses. He showed the influence of impure (laboratory) air on physiological processes and so came to the study of narcosis.

The University of Graz maintained its interest in bringing out new relations between anatomy and physiology which indeed were always related to one another. It was G. HABERLANDT who, stimulated by SCHWENDENER's views, turned himself successfully on a large scale to the development of physiological plant anatomy. His fundamental work on this subject (1884) is universally known, as is also his work on the physiological tissue systems of the plant. By carrying over the concepts of animal physiology to the elucidation of structure and function of plant tissues, he came to the discovery of plant sense organs, which serve in part for the perception of light, and in part for the reception of mechanical stimuli. The announcement of this generally interesting discovery, and likewise his book, *Eine botanische Tropenreise* (1893), made him well known far beyond the circle of his own colleagues. The work which he accomplished after his call to Berlin does not enter into the present discussion.

He was followed in the year 1911 by KARL LINSBAUER. It is noteworthy that again through his work there was developed an intimate connection between anatomy and physiology, to be sure in another sense than in HABERLANDT's work. The great work originated and edited by him, *Handbuch der Pflanzenanatomie* (since 1926), sought a deeper penetration of

histology with the results of cytological, physiological, and mechanico-developmental investigations. As a contribution to this work he personally contributed the chapter on *Epidermis*. In the forefront of his interests stands the physiology of irritability. In the movement of tendrils he established the fact that in this response a hitherto unrecognized significance is to be attributed to torsions. The movement of stomata is to him not so much a mechanical as an irritability movement. With the aid of modern recording methods he ascertained the rate of stimulus transmission in the primary petiole of *Mimosa pudica*, found that conduction of stimulus in this plant can take place in the vessels, and that the sieve tubes are not necessary for this function. A great part of LINSBAUER's efforts were devoted to the problem of developmental mechanics and general biology.

After the death of LINSBAUER, FRIEDL WEBER assumed his position. By chance it was ordained that again anatomical and physiological investigations were joined in his work in a still different way; physiological changes of the individual cells were brought into relation to certain changes in state of their protoplasm. The pursuit of this idea led to the founding of a "protoplasmatic anatomy." Through research in such a direction, "protoplasmatics" became in Austria the focus of attention.

In addition WEBER, in the journal *Protoplasma* which he founded, has created an international organ which serves the whole field of physical chemistry of the protoplast. In addition he shared in the editorial responsibility for the *Protoplasma Monographien*.

In a few words we will now mention the University of Innsbruck at which E. HEINRICHER was active for decades. His main subjects of research were *Viscum*, *Lathraea*, and green hemi-parasites whose germination and nutritional relations he cleared up in a series of investigations. SPERLICH is his successor.

In the exchange of scientific thought, Austria has always participated, but seldom, and only transitorily, have botanists from foreign lands come to the Austrian professional chairs; Austria has always contrived to meet its need of academic teachers and investigators from its own people. It is significant for the new march of progress of the home-land research that scientific life has not become set in the mold of tradition, but has developed as a living continuum through a half century in which the individuality of scholars insures the continuous flow of ideas. On the other hand, our land can point to the fact that it has given a group of important investigators to neighboring nations. Among these may be mentioned VON GUTTENBERG (Rostock), G. HABERLANDT (Berlin), BRUNO HUBER (Tharandt), O. RICHTER (Brünn), STRUGGER (Griefswald); and at the Yugoslavian University of Agram (now Zagreb), VALE VOUK is engaged.



# FOLIAR DIAGNOSIS: PRINCIPLES AND PRACTICE

WALTER THOMAS  
(WITH SEVEN FIGURES)

## Introduction

As a result of the broader view now taken with respect to the problems of soil fertility it is recognized that the conditions required for maximum growth must be sought from the facts of plant physiology as well as from those of soil science. For satisfactory growth a particular soil must satisfy certain conditions with respect to temperature, and to nutrient, water, and air supply to roots, the task of the investigator being to discover the best means of bringing the factors that govern these conditions under control. For this purpose the traditional chemical methods both of plant and of soil analyses have definite limitations; some have been abandoned, others are still in use.

In recent years interest has been renewed in the possibilities of the chemical analysis of the plant as a means of studying the nutrient relationships between the crop and the soil with respect to (1) the physiological requirement of a particular species and (2) the rate of supply of nutrients (10), *i.e.* the supplying power of the soil (27). It is well established that the composition of a particular plant species is profoundly influenced by the composition of the soil (40). Consequently, in order to determine the influence of the soil on the action of fertilizers by means of analysis of the plant it is necessary to experiment first with plants growing in a relatively homogeneous soil (*vide infra*) using systematically laid out plots differentially fertilized. When this is done, the next step is to ascertain the nature of the variation in the composition of plants of the same species and variety in similarly treated (duplicate) plots, the soils of pairs of which may show all types of variations.

There are many reasons, however, why the analysis of the entire plant is not the best means of approach toward the problem of control under field conditions. Apart altogether from the disadvantages of having to dig up and analyze whole plants, the results obtained by the gross analysis of a mass of heterogeneous organs possessing different functions are not a sufficiently sensitive comparative index in reflecting the responses of the plant to differences in its environment (soil and weather) (41). The classical investigations of PIERRE (34) pointed to the sensitivity of the leaves to changes in composition resulting from differences in environment. But as long as no care was taken to differentiate between leaves of different ages little progress was made. The presentation of the gross analysis of leaves of all ages of a plant species, frequently seen even in present day literature

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is of no service in diagnosis because neither the relative nor absolute amounts of the mineral entities are the same in leaves of different ages. Moreover, samples taken only at one period can give no information as to the course or nature of the changes in nutrition with growth in relation to differences in the medium. The further development of the method of leaf analysis ("diagnostic foliaire") as a means of studying the course of the absorption of mineral elements under the influence of different growth factors, especially those of fertilizer additions, is largely due to the labors of LAGATU and MAUME.

### Principles of the method of foliar diagnosis

Of the numerous methods employed to control the nutrition of plants under the conditions of practical agriculture only the method of foliar diagnosis has any serious claim to be based on physiological facts established by consistent experimental results obtained over a long period of years (15, 16, 17, 18, 19, 20, 21, 22, 26). The laws governing the facts are still obscure, although glimpses of their working have been revealed. Some of the facts and their implications have already been discussed in a series of papers by the writer (38, 39, 40, 41).

### FACTS UPON WHICH THE METHOD IS BASED

The experimental facts to which the method of foliar diagnosis is linked may be briefly summarized:

1. Two morphologically homologous leaves of the same species and variety are the seat of identical physiological processes when the medium (soil and meteorological factors) of the two is identical and, conversely, are the seat of physiological processes that are different when the medium is different (13, 18, 19, 21).

2. The composition of leaves of the same physiological (metabolic) age of plants grown on the same homogenous medium (soil and climatic) and receiving different fertilizer treatments reflects these differences in the sense that whenever a fertilizer element (whether N, P, or K) is effective, as determined by the response of the plant to that element, that response is always associated with an increase of that element in the dry foliage (18, 19, 37). This is not, as might appear at first, a principle *a priori*, because neither the processes of migration of the elements into the leaf from the stem nor export from the leaf into the stem is under consideration in the method of foliar diagnosis (*vide infra*), but only the amount of the element present in the leaf at the moment of sampling.

3. The composition—as determined by periodic analysis—of leaves of the same physiological (metabolic) age taken from plants of the same species growing on the same homogenous medium (soil and climatic) but receiving different fertilizer treatments are related to their development (9, 18, 42).

4. The magnitude of the variations in the composition, with respect to the dominant nutritive elements, of leaves of the same physiological age from plants of the same species grown in a homogenous soil and subjected either (1) to differential fertilizer treatment under the same meteorological conditions, or (2) to the resultant of meteorological factors acting in each particular growing season, is relatively quite large and, therefore, easily determinable. This delicate sensitivity of the leaf is no doubt related to its rôle as the laboratory of synthesis of the plant (41).

#### AN "IDEAL" SOIL NON-EXISTENT

Reference has been made to a "homogenous" (uniform) soil. Such an ideal soil, of course, does not exist in nature. If an area of land is divided into two plots, A and B, which are managed and fertilized alike and are sown at the same time with selected uniform seed, the yields may be different even when the land appears uniform to the eye. The yields may and, generally, will be widely different if the contour of one part differs greatly from the contour of the other, because the soil factors associated with differences in depth and texture will be different. If the land is fairly level and the soil uniform, however, the relative percentage-difference in yield  $\left(\frac{x-y}{x} \times 100\right)$  of two plots treated alike will generally not exceed 5 to 10 per cent., which limit for the purpose of the type of investigation we are considering in this paper is sufficiently close to discover and elucidate rules or principles.

#### DEFINITION OF FOLIAR DIAGNOSIS

Foliar diagnosis is characterized (18) as (a) the chemical composition of a leaf with respect to the dominant nutritive mineral entities at the instant of sampling, taken from a predetermined and suitable position on the stalk. (b) The foliar diagnosis for any given season (year) then will consist of a sequence of chemical states (composition) as determined under (a) on different dates, i.e., at different periods during the growth season. *The composition is based on the dry matter of the leaf without taking into consideration the weight of the dry material at each sampling or the number of leaves sampled from each plant.*

*It is clear, therefore, that no physiological significance can be attributed to the foliar diagnosis of any one fertilizer treatment (plot) considered alone. The method is comparative just as the method of analysis of entire plants is comparative (41). Foliar diagnosis considered independently of all other field data and of all other foliar diagnosis has no physiological significance.*

PROPER FORM TO EXPRESS MINERAL ELEMENT CONTENT.—The criticism by BARTHOLOMEW, WATTS, and JANSSEN (2) of the method used by the

writer of expressing the mineral content of a leaf on the basis of percentage of the element in the dried material is the result of misunderstanding and a confusion of thought. As JAMES (12) has well emphasized, the different ways of expressing the mineral content of plants in studies of mineral nutrition must be considered in relation to suitability. Each case must be considered on its merits. In the present instance, there can be no ambiguity nor question that the dry weight unit is the correct one. Furthermore, it is incorporated in the definition of foliar diagnosis (p. 573).

#### NOMENCLATURE

In investigations concerned with the mechanism of penetration into and translocation within the plant the logical procedure is to refer to all results in terms of the ions K, Ca, Mg,  $\text{NO}_3$ ,  $\text{H}_2\text{PO}_4$ , etc. In physiological investigations having a direct agronomical application, this method is not the most suitable one. The facts are that in practical agriculture and in the arts dependent upon it, a different mode and only one mode of expression is internationally used for the dominant fertilizer principles or entities. These are N for nitrogen,  $\text{P}_2\text{O}_5$  for phosphoric acid, and  $\text{K}_2\text{O}$  for potash. The writer has adopted this international nomenclature in this paper. And, as a matter of convenience, reference will frequently be made to the three dominant principles as "elements" or "entities." This should cause no confusion if the reader keeps in mind that all results are expressed as N,  $\text{P}_2\text{O}_5$ , and  $\text{K}_2\text{O}$ .

#### TWO METHODS OF EXPRESSING FOLIAR DIAGNOSIS GRAPHICALLY

The graphic presentation of the results of the periodic analyses of the leaves is important in the method of interpretation and may be done in one of two ways: (1) In terms of the percentage of the respective elements in the dried foliage as the ordinate and with the dates of sampling as the abscissa. (2) If only nitrogen, phosphoric acid, and potash are to be considered, the results may also be expressed graphically in trilinear coordinates.

The advantage of the first-mentioned method is that it possesses the capacity of expressing any number of elements for which analysis has been made and of showing the course of each independently. The second method is limited to three entities, in this case to nitrogen, phosphoric acid, and potash—elements which may properly be correctly considered as a unit of NPK, inasmuch as the content of all three in the dry foliage decreases with the age of the leaf, differing in this respect from Ca and Mg which accumulate with age.

**BASIC ASSUMPTION IN FERTILIZER PLOT EXPERIMENTS.**—The development and yield of a plant is, as already emphasized (p. 571), not exclusively dependent on nitrogen, phosphorus, and potassium. In drouth years water

would be the principal factor influencing yields. This factor and others associated with differences in soil texture will also enter in if the soil of the plots is not uniform. Consequently, there can be no *a priori* right to relate the development and yield to the composition of the leaf in terms of nitrogen, phosphoric acid, and potash. Nevertheless, fertilizer field-plot experiments are based on the assumption that the other factors for the plots to be compared are nearly equal. When they are not, recourse is had to many replicates. The present trend is to plan field plots in such a way that each experiment gives a measure of its own error, although, of course, they can never be exact.

#### QUANTITATIVE INDEX EXPRESSING EFFECT OF A FERTILIZER ENTITY

It has been a tradition to define the response to different fertilizer treatments in terms of the fertilizer or salts used. This point of reference has been used not only by the practical agriculturists but also by mathematicians (7), physiologists (8), and by agronomists generally. But when the fertilizer (or nutrient salt) treatment is used as the point of reference it is difficult to ascertain whether the effect is due to the sum of the individual elements (ions) or to an effect proportional to their product or even to a more complicated function of their individual effects (7, 8, 14).

If, however, a more rational base of reference be taken, *viz.*, the composition of the plant or better its "reflect," *viz.*, a leaf suitably chosen, the problem is considerably simplified. On this basis we may define without ambiguity the quantitative index expressing the effect of any one fertilizer element at the moment of sampling the leaves from any one plot, as the ratio of the amount of that element contained in the leaves of the plant growing on the plot considered, to the amount of that element in the homologous leaves from a plant growing on a plot receiving no fertilizer, sampled at the same time. Likewise, the quantitative index, expressing the effect of any combination of the elements present in the fertilizer used in any plot at the moment of sampling the leaves, may be defined as the ratio of the sum of the elements considered, contained in the leaves of plants growing on this plot at the moment of sampling, to the sum of these elements in morphologically homologous leaves of plants growing on the unfertilized plot.

#### CONCEPTS OF QUANTITY AND QUALITY OF NUTRITION

Inherent in the expression of the analyses in terms of the dried foliage are two concepts, *viz.*, that of *quantity* and *quality* of the mineral constituents. The concept of *quantity* or intensity of nutrition of the selected leaves consists of the sum ( $N + P_2O_5 + K_2O$ ) of each element at the moment of sampling expressed as a percentage of the dried material. The *quality* of nutrition is the ratio of these entities to each other at the moment of sampling.



## COMPOSITE NPK-UNIT

EXPRESSING THE THREE DOMINANT FERTILIZER PRINCIPLES AS A UNIT.—The quantities of nitrogen, phosphoric acid, and potash in the dried foliage are expressed as a unit (23, 24) as follows:

Let  $x$  be the percentage of N,  $y$  the percentage of  $P_2O_5$ , and  $z$  the percentage of  $K_2O$  in the dry material of the leaf at the moment of sampling.

Let

$$s = x + y + z. \quad (1)$$

Then  $s$  denotes a quantity of mineral nutrients supplied by N,  $P_2O_5$ , and  $K_2O$  in proportion to the amounts  $x$ ,  $y$ , and  $z$  present in the leaf considered at the moment of sampling. Dividing by  $s$  we get

$$1 = \frac{x}{s} + \frac{y}{s} + \frac{z}{s}. \quad (2)$$

where  $\frac{x}{s}$ ,  $\frac{y}{s}$ , and  $\frac{z}{s}$  represent fractions which indicate the proportion contributed by N,  $P_2O_5$ , and  $K_2O$ , respectively, to a unit quantity of the three elements. Let  $\frac{x}{s}$  be denoted by  $x_1$ ,  $\frac{y}{s}$  by  $y_1$ , and  $\frac{z}{s}$  by  $z_1$ . Substituting in (2), we get

$$x_1 + y_1 + z_1 = 1. \quad (3)$$

The quantity  $x_1 + y_1 + z_1$  represents the composition of the leaf in terms of the proportions in which nitrogen, phosphoric acid, and potash are present in unit quantity of the leaf considered at the moment of sampling and, consequently, it is an expression giving the physiological ratios between N,  $P_2O_5$ , and  $K_2O$  in the synthetic laboratory of the leaf. It should be emphasized that this unit is independent of the total quantity  $s$  of N,  $P_2O_5$ , and  $K_2O$  in the dry material of the leaf. *The quantity "s," therefore, is a magnitude expressing the quantity of nutrition at the moment of sampling the leaf.*

The magnitude denoted by  $s$  indicates the number of units of nitrogen, phosphoric acid, and potash contained in 100 parts of dry matter and, consequently,  $s$  expresses the *quantity* (intensity) of nutrition with respect to nitrogen, phosphorus, and potassium. At first blush it might not seem justified to combine into one unit quantities as heterogeneous as N,  $P_2O_5$ , and  $K_2O$ . However, since the chemical reactions involved are not considered in this expression, but only the amounts of these fertilizer principles in the leaf irrespective of their chemical rôle, and inasmuch as the entities N,  $P_2O_5$ , and  $K_2O$  are those used in practical agriculture, it would be incongruous to express the characteristic *intensity of nutrition* in milligram equivalents.

Although these two magnitudes representing the *quality* and *quantity*

of nutrition are abstractly distinct, they are inseparably connected in the physiological processes of the leaf. They must, therefore, be considered simultaneously when relating the nutrition with respect to NPK of a leaf and its physiological effect on the development of the plant.

DIAGRAMMATIC ILLUSTRATION OF NPK-UNIT CONCEPT.—These concepts may be illustrated in the following manner (25).

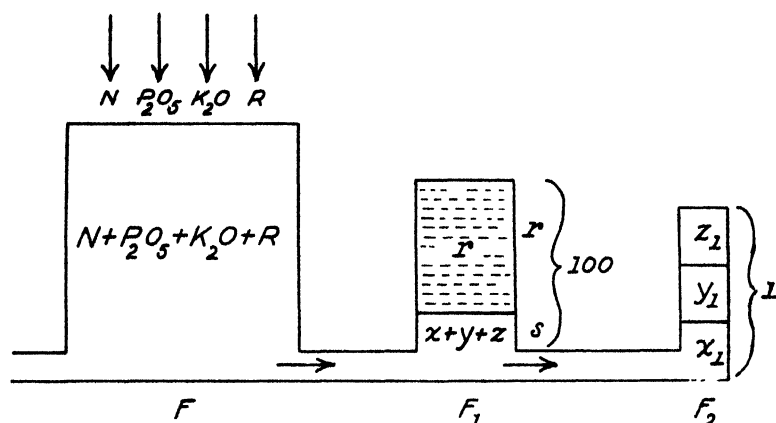


FIG. 1. Diagrammatic representation of the concept of the method of foliar diagnosis.

In figure 1 let  $F$  represent a leaf into which are migrating substances containing N, P, K plus other materials (R). Of the substances entering the leaf a portion will be elaborated, and a portion will remain in an unelaborated form. Let the quantities of all nutrients present at the particular instant of sampling a leaf be represented by a communicating vessel  $F_1$  of capacity 100, of which  $x + y + z = s$  represents the quantities of  $N + P_2O_5 + K_2O$  in 100 parts of the dried foliage and  $r$  the other substances present also in percentage of the dried foliage.  $F_1$  represents the type of investigation with which foliar diagnosis is concerned.  $F_1$  may then be regarded as an indicator of  $s$ .

Let us now imagine another vessel ( $F_2$ ) of unit capacity to be connected with  $F_1$ , completely filled at the instant considered, and into which only N,  $P_2O_5$ , and  $K_2O$  are entering and in the same proportion as they are present in  $F$  and, consequently, also in  $F_1$ . Let us also suppose that N,  $P_2O_5$ , and  $K_2O$  are separated in  $F_2$  into three layers as shown in the diagram. Then the sum of the quantities  $x_1, y_1, z_1$  of nitrogen, phosphoric acid, and potash, respectively, of  $F_2$  is always equal to 1. The vessel  $F_2$  may then be regarded as the indicator of  $x_1, y_1, z_1$ , where  $x_1 = \frac{x}{s}$ ,  $y_1 = \frac{y}{s}$ , and  $z_1 = \frac{z}{s}$ .

CALCULATION OF NPK-UNIT IN TERMS OF MILLIGRAM EQUIVALENTS.—The *quality* factor of nutrition, i.e., the proportion (ratio) of elements

found in the leaf at the respective sampling dates, is most suitably expressed as milligram equivalents (M.E.), because this factor—quality—must be dependent upon and related to the chemical reactions involved. These are calculated as follows:

Let  $M_x$  be the percentage of N,  $M_y$  the percentage of  $P_2O_5$ , and  $M_z$  the percentage of  $K_2O$  in the leaf at the moment of sampling. Then, reducing to milligram equivalents:

$$E_x = 1000 \frac{M_x}{N}; \quad E_y = 1000 \frac{M_y}{\frac{1}{2}(P_2O_5)}; \quad E_z = 1000 \frac{M_z}{\frac{1}{2}(K_2O)}. \quad (4)$$

Let  
Dividing by  $S$ , we get

$$1 = \frac{E_x}{S} + \frac{E_y}{S} + \frac{E_z}{S}. \quad (5)$$

Then  $\frac{E_x}{S}$ ,  $\frac{E_y}{S}$ ,  $\frac{E_z}{S}$ , respectively, represents a fraction which indicates in milligram equivalents the proportional parts of N,  $P_2O_5$ ,  $K_2O$  in a unit quantity of the three elements.

$$\begin{aligned} \text{Let } \frac{E_x}{S} \text{ be denoted by } x^1, \text{ then } E_x &= x^1 S \\ \text{“ } \frac{E_y}{S} \text{ “ “ “ } y^1, \text{ “ } E_y &= y^1 S \\ \text{“ } \frac{E_z}{S} \text{ “ “ “ } z^1, \text{ “ } E_z &= z^1 S \\ x^1 + y^1 + z^1 &= 1. \end{aligned} \quad (6)$$

The quantity  $x^1 + y^1 + z^1$  represents the composition of the NPK-unit of the leaf at the moment of sampling in terms of milligram equivalents.

To avoid fractional quantities it is convenient to multiply the terms of equation (6) by 100. Then

$$100 x^1 + 100 y^1 + 100 z^1 = 100.$$

$$\text{Let } X = 100 x^1, \quad Y = 100 y^1, \quad Z = 100 z^1.$$

$$\text{Then } X + Y + Z = 100. \quad (7)$$

$X + Y + Z$  is denoted the composite NPK-unit or, for simplicity, the NPK-unit. For each simultaneous value of  $X$ ,  $Y$ ,  $Z$  there is one point and only one on an equilateral triangle each of whose sides = 100.

READING THE TRILINEAR COORDINATE GRAPHS.—The higher the locus of a coordinate point moves in the direction of the summit of the triangle representing  $N = 100$  per cent. in the NPK-unit, the greater is the proportion of nitrogen in this unit. Likewise, the movement of the locus of a coordinate point toward the left base-apex of the triangle representing  $K_2O = 100$  per

cent. in the *NPK-unit*, the greater the proportion of potash in this unit. Similarly, changes in the direction of the locus of a point towards the right base-apex of the triangle shows that  $P_2O_5$  in the *NPK-unit* has increased.

### Application of method

#### PLOTS WITH TREATMENTS USED

The potato plants used in this investigation were grown on tier 1, section B, of the Vegetable Fertility Plots of the Department of Horticulture. These plots were laid out in 1916. The description of the experimental lay-out given by MACK (29) is in part as follows:

"The area is laid out in four sections, 245.8 feet wide and 308.4 feet long. . . . Each of the sections is divided into six tiers. . . . The tiers are further divided into seventeen plots, the dimensions of which are 12 by 36.3 feet, and which are separated from each other by guard strips 6 feet wide. The area of the plots, exclusive of guard strips, is exactly 1/100 of an acre."

The four-year rotation consists of cabbage, potatoes, tomatoes, and sweet corn with timothy and rye as a green-manure crop.

The plots examined in this paper together with their treatments and yields (in 1935) are given in table I.

TABLE I  
PLOTS WITH NUMBERS AND TREATMENTS

TIER	PLOT NO.	COMMERCIAL FERTILIZER APPLIED	COMMERCIAL FERTILIZER APPLIED TO PLOT	N, $P_2O_5$ AND $K_2O$ EQUIVALENT	SYMBOL USED	YIELDS PER PLOT
			<i>lb.</i>	<i>lb.</i>		<i>lb.</i>
1	2	Sodium nitrate	4.0	0.6	N	109
1	3	Superphosphate	6.25	1.0	P	114
1	4	Potassium chloride	1.666	0.8	K	155
1	6	Sodium nitrate	4.0	0.6	NP	124
		Superphosphate	6.25	1.0		
1	7	Sodium nitrate	4.0	0.6	NK	163
		Potassium chloride	1.666	0.8		
1	8	Superphosphate	6.25	1.0	PK	148
		Potassium chloride	1.666	0.8		
1	10	Sodium nitrate	4.0	0.6	NPK	162
		Superphosphate	6.25	1.0		
		Potassium chloride	1.666	0.8		

The complete fertilizer then has a ratio of  $N:P_2O_5:K_2O$  of 0.6:1.0:0.8 or 6:10:8.

The plots of tier 1, section B, examined in this experiment were relatively uniform within the limitations defined on page 573. These plots represent only a few of the fertilizer combinations used in these crop fertility experiments. Other amounts with the same and also with different ratios are applied to the remaining plots. The best treatments reported in this paper, *viz.*, NK and NPK, are not those which have given the highest yields in this long continued experiment. The highest yield obtained with commercial fertilizers has been secured by a complete fertilizer containing the same quantity of nitrogen and potash, but containing 50 per cent. more phosphoric acid.

#### SELECTION AND SAMPLING OF LEAVES

**IDEAL SAMPLE.**—The ideal sample at any selected sampling date would consist of leaves of the same rank—(physiological or metabolic age)—on the stalks taken at the same time from a sufficient number of plants in a row along the plot to give a sample representative of the plot. Each successive row would then be sampled in like manner on each of the successive sampling dates. The leaf chosen from a plant would conveniently be the one immediately above the caducous leaves at the base of a stalk.

**SAMPLING IN RELATION TO SENESCENCE.**—Under practical field conditions one must determine beforehand the rank of the leaf or leaves to be selected, for, as explained earlier in this paper (pp. 573, 574), the method consists essentially of a comparison of the mode of nutrition of leaves of the same metabolic or physiological age from plants growing on plots subjected to different factors. To this end the longer the period of observation the greater will be the information obtained with respect to the differences in the course (nature) of the nutrition in response to the different treatments. If two plots receiving different treatments are planted the same day, the plants growing thereon will not reach maturity at the same time. At any intermediate date the plants considered will be at different stages of their life cycle, which can be determined in a general way by observation. With the qualification to be made later, the shorter-lived plants will show the greater amount of color change—from green to yellow and then brown. In the case of the potato leaf a stage is reached at which a golden yellow appears, followed by a further and final color change when it shrivels and becomes a dark brown and shortly after which it drops from the stem. These color changes are in general an indication of their metabolic age and the proportion of yellow and withered leaves on the plant is an index of condition.

**CAUSES OF SENESCENCE.**—The aging of leaves has been associated with the disappearance of potassium (3, 5), of nitrogen (4), of water (6), and of all three elements, N, P, and K, simultaneously (36). The fact that an in-

creased supply of K has been found to delay breakdown of the mechanism causing aging (11) and that at higher concentrations this balance is under certain conditions upset, when yellowing becomes as fast or faster than with a deficiency (11), indicates that the mechanism is conditioned by physiological balance. In many instances, cause cannot be distinguished from effect (3).

**METHOD OF SAMPLING.**—Only under the practical field conditions of potato growing, where plants are planted in rows relatively close together and are well nourished, is the problem of sampling in relation to senescence of much moment. In such plants leaves higher up than the second and third may commence to wither before the end of the allotted sampling dates. This difficulty may be overcome in one of two ways. The one would be to take samples of the second or (and) third leaf from a stalk at short intervals of time. Then when the plants are sufficiently developed to take *simultaneously* samples of higher rank on the stalk, *e.g.*, the seventh and eighth leaf, and finally, as the plant matures, leaves still higher, *e.g.*, the twelfth or thirteenth leaf. This method of sampling has the advantage of giving three sets of overlapping samples which, if the plants are not subjected to too violent external disturbances, may be regarded as of corresponding successive physiological ages. The three sets of samples must then be treated separately in reporting results. This method will be illustrated in a later paper.

The second method of overcoming the difficulty is to sample a leaf from a particular position higher on the stalk, *e.g.*, the fourth or fifth leaf, which would give reasonable assurance of enabling observations to be made, say at three or four dates at intervals of about 12 to 20 days. The exact interval between samplings is not important. In the case of the potato plant the writer has found that there is at any given intermediate stage of maturity a progressive increase in nitrogen and phosphoric acid and a progressive decrease in potash of the dry matter of the leaf as one proceeds from the base upward, *i.e.*, the younger the leaf is. (*Cf.* MACGILLIVRAY, 28, JAMES and PENSTON, 13, and PENSTON, 32.)

Accordingly, in the latter method of sampling there will be a slight progressive shift in rank on the stalk of the leaf of that rank with time. However, the differences expressed as percentages of the dried foliage with respect to N,  $P_2O_5$ , and  $K_2O$  of two consecutive leaves on the stalk of all cultivated plants thus far investigated have been found to be relatively insignificant compared with the differences between the composition of leaves of the same or approximately the same physiological age from plots receiving different treatments. This has been found to be true for any two successive leaves of the potato plants in these experiments. Such differences, therefore, for the purpose of this type of inquiry may be neglected.

Diurnal variations exist in the content of nitrogen, phosphoric acid, and

potash in the leaves of the potato (33). The magnitude of these diurnal variations is also insignificant compared with the magnitude of the changes considered in the method of foliar diagnosis. Samples, however, should be taken during the same day.

In the present experiments leaves were sampled on four different dates during July and August, and from consecutive rows on each successive date from two stems issuing from the mother tuber from each of the fourteen plants in a row. The number of samplings is arbitrary but at least three samplings should be made. The number of leaves sampled is also immaterial but, obviously, should not vary too greatly from one plot to another. The development of the plants sampled from each plot should be regular and homogeneous, for unless this is the case there would be no justification for considering the samples as representing an average sample.

*It should now be clear that foliar diagnosis must be expressed in terms of the rank of the leaf on the stem, the variety of plant, and the year in which samples were taken.*

#### REMOVAL OF SPRAY RESIDUES, SOIL, AND DUST FROM LEAVES

The spray materials used contained calcium (and magnesium). It is desirable to remove as much spray material from the leaves as possible, including any soil and dust adhering thereto, so as not to affect the weight of the dry material taken for analysis. The soil and dust are easily removed and most of the spray materials also by brushing with a stiff brush in the field immediately after the samples are taken.

#### METHOD OF DRYING LEAVES

The leaves were dried in a drying oven at 100° C. after being brought in from the field and then finely ground in a Wiley mill.

#### DETERMINATION OF NITROGEN, PHOSPHORIC ACID, AND POTASH

Nitrogen was determined by the Kjeldahl-Gunning method to include the nitrogen of nitrates (1), phosphoric acid by the RICHARDS and GODDEN'S (35) modification of the PEMBERTON-NEUMANN (30, 31) method, and potash by the Lindo-Gladden method (1).

#### Presentation of results

Table II gives the results of the analysis of the fourth and fifth leaves expressed in (a) percentage of N,  $P_2O_5$ , and  $K_2O$  in the dried foliage, (b) milligram equivalents, and (c) the NPK-unit.

#### CALCULATION OF LOCI OF NPK-UNIT

The following illustrates the method of calculating a coordinate point:

TABLE II

COMPOSITION OF LEAVES OF RANK 4 AND 5 AT PERIODIC INTERVALS IN TERMS OF PERCENTAGE OF N, P<sub>2</sub>O<sub>5</sub>, AND K<sub>2</sub>O IN DRIED FOLIAGE, MILLIGRAM EQUIVALENTS, AND THE NPK-UNIT

DATE OF SAMPLING	DRIED FOLIAGE				MILLIGRAM EQUIVALENTS				COMPOSITION OF THE NPK-UNIT			
	N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O		N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	E <sub>x</sub> + E <sub>y</sub> + E <sub>z</sub>	X	Y	Z	
	(M <sub>x</sub> )	(M <sub>y</sub> )	(M <sub>z</sub> )		(E <sub>x</sub> )	(E <sub>y</sub> )	(E <sub>z</sub> )	(S)	$\left(100 \frac{E_x}{S}\right)$	$\left(100 \frac{E_y}{S}\right)$	$\left(100 \frac{E_z}{S}\right)$	
	%	%	%		mg. eq.	mg. eq.	mg. eq.	mg. eq.	%	%	%	%
					N plot (tier 1, no. 2)							
July 7	5.12	.404	3.97		365.568	17.089	84.561	467.218	78.244	3.658	18.098	
July 29	4.42	.534	2.54		315.388	22.588	54.102	392.278	80.450	5.759	13.791	
Aug. 9	4.15	.472	2.01		296.310	19.966	42.813	359.089	82.517	5.561	11.922	
Aug. 24	3.64	.344	1.77		259.896	14.551	37.701	312.148	83.261	4.661	12.078	
					P plot (tier 1, no. 3)							
July 7	4.57	.550	2.41		326.298	25.042	51.333	402.673	81.034	6.218	12.747	
July 29	3.46	.521	1.64		247.044	22.038	34.932	304.014	81.260	7.250	11.490	
Aug. 9	3.24	.520	1.29		231.336	21.996	27.477	280.809	82.383	7.831	9.786	
Aug. 24	2.79	.450	1.11		199.206	19.035	26.643	241.884	82.359	7.868	9.773	
					K plot (tier 1, no. 4)							
July 7	4.61	.424	6.32		329.154	17.935	134.573	481.662	68.336	3.725	27.939	
July 29	3.87	.540	5.22		276.318	23.842	111.122	410.282	67.349	5.567	27.084	
Aug. 9	3.39	.464	4.91		242.046	19.627	104.689	366.362	66.068	5.358	28.575	
Aug. 24	2.78	.370	5.00		198.492	15.651	106.500	320.643	61.904	4.881	33.215	



TABLE II—(Continued)

DATE OF SAMPLING	DRIED FOLIAGE				MILLIGRAM EQUIVALENTS					COMPOSITION OF THE NPK-UNIT				
	N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O		N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	E <sub>x</sub> + E <sub>y</sub> + E <sub>z</sub>		X	Y	Z		
	(M <sub>x</sub> )	(M <sub>y</sub> )	(M <sub>z</sub> )	%	(E <sub>x</sub> )	(E <sub>y</sub> )	(E <sub>z</sub> )	(S)		(100 $\frac{E_x}{S}$ )	(100 $\frac{E_y}{S}$ )	(100 $\frac{E_z}{S}$ )		
	%	%	%		mg. eq.	mg. eq.	mg. eq.	mg. eq.		%	%	%		
NP plot (tier 1, no. 6)														
July 7	5.14	.590	2.53		366.996	24.957	53.910	445.863		82.311	5.598	12.091		
July 29	3.87	.532	1.52		276.318	22.504	32.355	331.177		83.435	6.794	9.771		
Aug. 9	3.71	.486	1.19		264.894	20.558	25.262	310.714		85.253	6.617	8.130		
Aug. 24	3.36	.390	1.09		239.904	16.751	23.110	279.765		85.752	5.987	8.261		
NK plot (tier 1, no. 7)														
July 7	5.10	.464	6.63		364.140	19.627	141.176	524.943		69.367	3.739	26.894		
July 29	4.03	.510	5.55		287.742	21.573	118.300	427.615		67.290	5.045	27.665		
Aug. 9	3.78	.452	5.28		269.892	19.119	112.443	401.454		67.229	4.763	28.008		
Aug. 24	3.26	.352	5.46		232.764	14.889	116.234	363.887		63.964	4.092	31.941		
PK plot (tier 1, no. 8)														
July 7	4.55	.620	6.76		324.870	26.395	143.967	495.232		65.599	5.330	29.071		
July 29	3.36	.510	5.32		239.904	21.573	113.337	374.814		64.006	5.755	30.239		
Aug. 9	3.08	.490	5.35		219.912	20.980	113.912	354.804		61.981	5.913	32.105		
Aug. 24	2.82	.430	5.04		201.348	18.189	107.309	326.846		61.603	5.565	32.832		
NPK plot (tier 1, no. 10)														
July 7	4.98	.584	6.59		355.572	24.703	140.346	520.621		68.297	4.744	26.958		
July 29	3.88	.509	4.34		277.032	21.531	92.378	390.941		70.863	5.507	23.630		
Aug. 9	3.62	.482	4.70		258.468	20.389	100.046	376.903		68.215	5.381	26.405		
Aug. 24	3.14	.392	4.35		224.196	16.582	92.612	333.390		67.249	4.973	27.778		

The analytical results (table II) for the N plot (no. 2) sampled July 7 are  $N = 5.12$  per cent.,  $P_2O_5 = 0.404$  per cent., and  $K_2O = 3.97$  per cent.

The *intensity of nutrition* (s) of the selected leaves for the N plot on July 7 is, therefore,

$$N + P_2O_5 + K_2O = 5.12 + 0.404 + 3.97 = 9.494$$

To evaluate the *NPK-unit* the percentage values are converted (pp. 577, 578) into milligram equivalents (M.E.) of N,  $P_2O_5$ , and  $K_2O$  as follows:

$$E_x = 1000 \times 0.0714 \times 5.12 = 365.568 \text{ M.E. of N}$$

$$E_y = 1000 \times 0.0423 \times 0.404 = 17.089 \text{ M.E. of } P_2O_5$$

$$E_z = 1000 \times 0.213 \times 3.97 = 84.561 \text{ M.E. of } K_2O$$

Hence

$$S = E_x + E_y + E_z = 365.568 + 17.089 + 84.561 = 467.218$$

and

$$X = 100 \frac{E_x}{S} = \frac{100 \times 365.568}{467.218} = 78.244$$

$$Y = 100 \frac{E_y}{S} = \frac{100 \times 17.089}{467.218} = 3.658$$

$$Z = 100 \frac{E_z}{S} = \frac{100 \times 84.561}{467.218} = 18.098.$$

These values give the three coordinate points required. It should be noted that  $78.244 + 3.658 + 18.098 = 100$ . We then have for each simultaneous value of  $X, Y, Z$  a representative point and only one upon the surface of an equilateral triangle the sides of which are expressed in 100 units.

#### METEOROLOGICAL CONDITIONS DURING 1935 GROWING SEASON

The foliar diagnosis specified in this paper is that for the fourth and fifth leaves for the potato variety Rural Russet for the year 1935. As a matter of record the meteorological conditions (rainfall and mean daily temperature) for the month preceding the sampling and for the intervals between samplings are given in table III.

TABLE III  
RECORD OF METEOROLOGICAL CONDITIONS

DATE	RAINFALL	MEAN DAILY TEMPERATURE
	<i>in.</i>	<i>°F.</i>
June 1-30 .....	3.88	66.5
July 6-29 .....	5.67	73.5
July 30-Aug. 9 .....	1.19	70.8
Aug. 10-24 .....	0.35	71.4

### Interpretation and discussion of results

INDICATIONS GIVEN BY FIRST METHOD OF GRAPHIC PRESENTATION BASED ON PERCENTAGES OF ELEMENTS IN DRIED FOLIAGE AS ORDINATE AND DATES OF SAMPLING AS ABSCISSA

When the percentage of an element in the dried foliage at a particular sampling date is plotted as the ordinate and the dates of sampling as the abscissa, as described in the first method (p. 574), the relationships between the composition of the leaves, with increasing age, from plants growing on the respective plots are clearly brought out and are shown in figure 2.

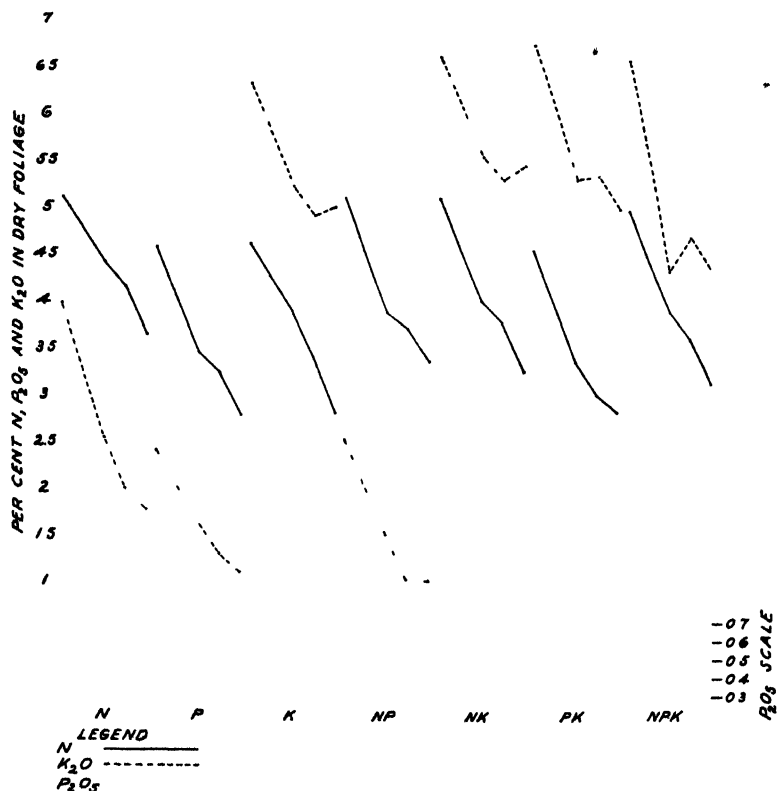


FIG. 2. Periodic analysis of the fourth and fifth leaves of potato plants growing on the differently treated plots showing percentage of elements in the dried foliage as ordinates and the dates of sampling as abscissa.

The indications given by the graphs are summarized below:

**NITROGEN GRAPHS.**—The content of nitrogen expressed as percentage in the dried foliage of plants which received mineral nitrogen applications is higher throughout the whole period than in those which received none.

Furthermore, the nitrogen content decreases with the increasing age of the leaf throughout the whole period in all plots.

**PHOSPHORIC ACID GRAPHS.**—The content of phosphoric acid expressed as a percentage in the dried foliage from plants which received phosphate applications is higher throughout the whole period than in those which received none. The phosphoric acid content decreases with the increasing age of the leaves of plants growing on plots to which mineral phosphate was added; but in the leaves of all plants which did not receive mineral phosphate addition a maximum occurs at the second sampling (July 29), after which the content of  $P_2O_5$  again decreases regularly with the increasing age of the leaf. This accumulation is undoubtedly a temperature effect. The curve of mean daily temperature is an ascending one up to July 24, after which it begins to descend. This temperature effect would, however, be masked in the plots where phosphorus is rapidly utilized.

**POTASH GRAPHS.**—The content of potash expressed as a percentage in the dried foliage of all plants which received mineral potash applications is very much higher than in those which did not. The potash content decreases regularly throughout the whole period with increasing age of the leaves of plants growing on plots which did not receive mineral potash. But accumulation has occurred in the leaves from all plants growing on plots which received mineral potash applications. This accumulation commenced in some cases (NPK, no. 10; PK, no. 8) between July 29 and August 9 and in others (NK, no. 7; K, no. 4) between August 9 and August 23.

JAMES (12) concludes that in the potato plant potassium is eventually heaped up considerably in excess of the amounts which are necessary to maintain the declining rate of growth. The data presented above show that generalizations with respect to accumulation of potassium are not possible. This accumulation is the result of a lack of physiological balance. It will be shown in a later paper that this accumulation of potassium has not occurred in the leaves of plants growing on plots which received sufficient nitrogen and phosphoric acid for the utilization of the potash.

It is thus seen that periodic analysis of leaves of the same physiological age from plants growing on plots treated with different fertilizers reflects these additions. This also holds true for plots on tier 2 and their duplicates on tiers 4 and 5. The graphs show by their position and their form the nature or course of the nutrition with respect to nitrogen, phosphorus, and potassium in the plants growing on the different plots. The steepness (slope) of a graph of a particular element indicates the relative demand in relation to the supply of that element.

Certain other indications furnished by the data given in table II are best brought out by the second method described on pages 576 and 577. In this method nitrogen, phosphoric acid, and potash are evaluated as a unit-NPK

and the results are plotted in trilinear coordinates. This method is illustrated in figures 4, 5, 6, and 7.

INDICATIONS GIVEN BY SECOND METHOD OF GRAPHIC PRESENTATION IN  
WHICH RESULTS ARE EXPRESSED AS NPK-UNITS AND PLOTTED  
IN TRILINEAR COORDINATES

In interpreting these graphs (figs. 4, 5, 6, 7) it should be kept in mind that a fertilizer may intervene to produce either (1) an increase in the sum ( $N + P_2O_5 + K_2O$ ), i.e., in the *intensity of nutrition*, or (2) a change in the composition of the *NPK-unit*, or (3) a change in both (1) and (2) simultaneously.

The *intensities of nutrition* are shown in figure 3 with the percentage of

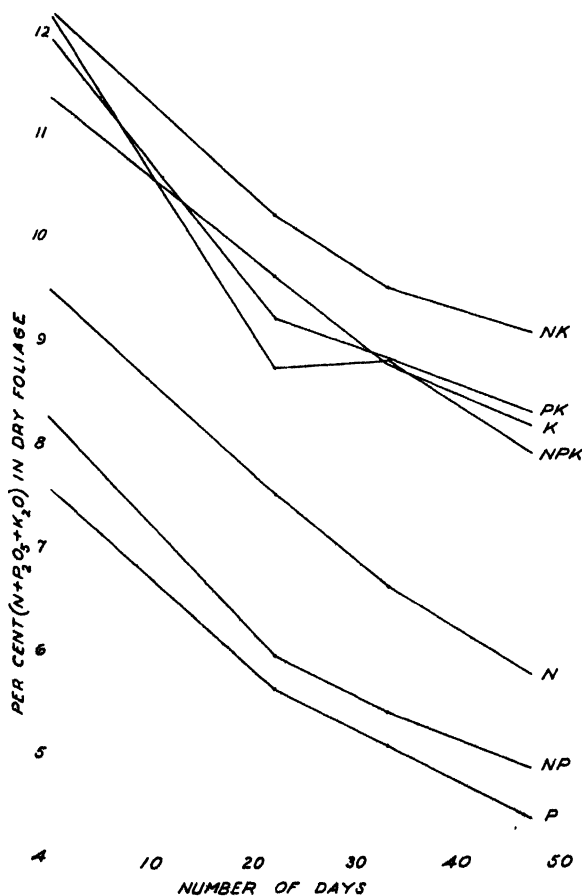


FIG. 3. Relationships of the intensities of nutrition of plants growing on differently treated plots.

TABLE IV

RELATION OF MEAN NPK UNIT AND MEAN INTENSITY OF NUTRITION AT FOUR SAMPLING DATES TO YIELDS OF POTATOES ON VARIOUS PLOTS

TREATMENT	TIER	PLOT	MEAN NPK-UNITS (MEAN OF 4 SAMPLINGS)					INTENSITY OF NUTRITION (MEAN OF 4 SAMPLINGS)	YIELDS			CLASS ORDER
									POUNDS PER PLOT	RELATIVE YIELDS NK = 100		
			N	:	P <sub>2</sub> O <sub>5</sub>	:	K <sub>2</sub> O					
			%	%	%	%		lb.	%			
NK	1	7	66.92	:	4.28	:	28.61	10.24	163	100.00	1	
NPK	1	10	68.65	:	5.15	:	26.19	9.39	162	99.38		
K	1	4	65.91	:	4.88	:	29.20	9.48	155	95.09	2	
PK	1	8	63.29	:	5.64	:	31.06	9.56	148	90.80		
NP	1	6	84.18	:	6.25	:	9.56	6.10	124	76.07	3	
P	1	3	81.76	:	7.29	:	10.95	5.65	114	69.99		
N	1	2	81.12	:	4.91	:	13.97	7.34	109	66.93		

$N + P_2O_5 + K_2O$  in the dried foliage from plants growing on the respective plots as the ordinate and as abscissa the dates of sampling. The coordinate points for the respective sampling dates, July 7 and 29, and August 9 and 24, are shown by dots. The displacement in a particular graph from one sampling date to another indicates the changes in the *intensity of nutrition* with the age of the leaves of the plants growing on the plot considered; and the relationship in form and position of the graphs to one another indicates the relative intensities of nutrition (s) resulting from the different treatments.

In figures 4, 5, 6, and 7 are shown in trilinear coordinates the changes in the composition of the *NPK-unit* (i.e., in the *quality of nutrition*) of the selected leaves from plants growing on the differently treated plots. The numbers 1, 2, 3, 4 at the respective coordinate points indicate the successive sampling dates, July 7 and 29, and August 9 and 24, respectively. The displacement of a point from one sampling date to another, therefore, indicates the course of the nutrition with respect to the NPK-unit, as previously defined (p. 578), and which represents the equilibrium between  $N$ ,  $P_2O_5$ , and  $K_2O$  at the moment of sampling.

RELATIONSHIP BETWEEN FOLIAR DIAGNOSIS AND YIELDS OF TUBERS.—  
*The mean NPK-unit.*—Table IV gives (1) the mean NPK-units for each plot, i.e., the mean NPK-unit for the four sampling dates of the selected

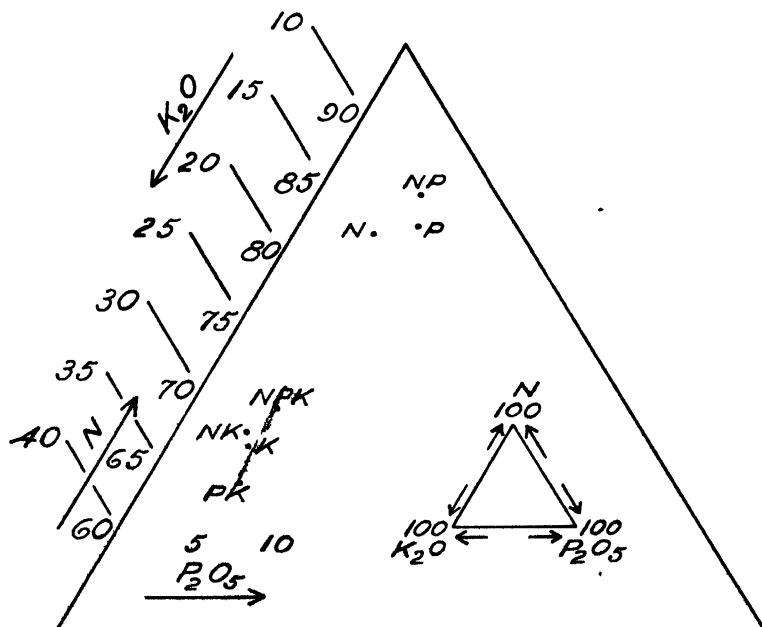


FIG. 4. Relative positions of the mean NPK-units.

leaves from plants growing on each plot; (2) the mean intensities of nutrition, and (3) the actual and relative yields of tubers on the basis of the highest yielding of the plots examined being placed at 100.

The mean NPK-unit for each plot is shown graphically in figure 4 by means of a single point for each plot. Each point, therefore, corresponds to the center of gravity (C.G.) of the detailed diagram shown in figures 5, 6, and 7 for the corresponding plot.

The plots are seen to fall into two groups according to their foliar diagnosis, *i.e.*, to their position on the triangle. The one group consisting of the N, P, and NP plots which are found high up toward the N = 100 per cent. apex of the triangle (*i.e.*, the corner representing N = 100 per cent.), and the other group the plots NK, NPK, K, and PK which are found much lower down in the triangle toward the left corner, *i.e.*, the corner representing  $K_2O = 100$  per cent.

The latter group can be further subdivided into two groups according to the extent of their displacement toward the base of the triangle. The points representing, respectively, the mean NPK-unit of the plots K (no. 4) and PK (no. 8) are lower than the points representing the plots NK (no. 7) and NPK (no. 10). The groups are designated as follows:

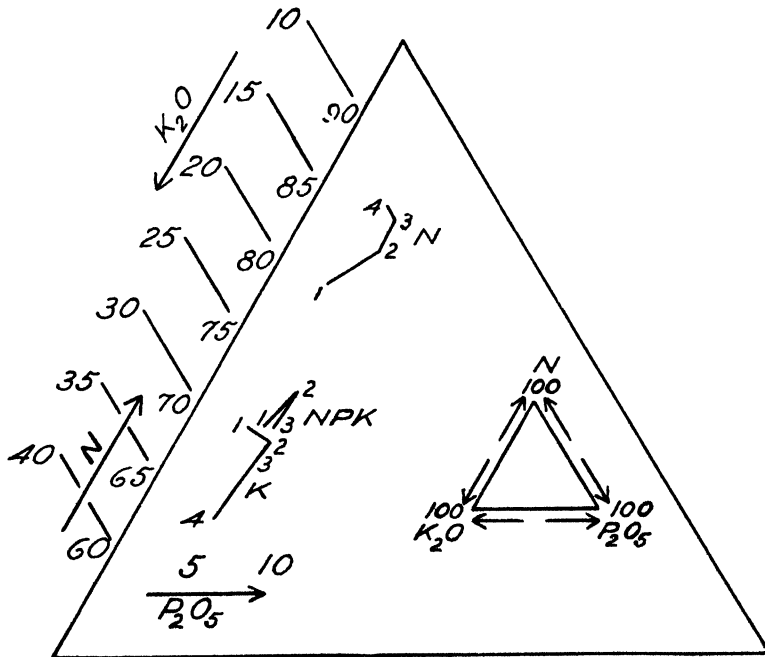


FIG. 5. Course of nutrition of plants growing on the N (no. 2), K (no. 4), and NPK (no. 10) plots as indicated by the changes in their NPK-units from one sampling date to another.



Group 3, NP (no. 6), P (no. 3), N (no. 2), having low yields.

Group 2, K (no. 4), PK (no. 8), having intermediate yields.

Group 1, NK (no. 7), NPK (no. 10), having high yields (the highest reported in this paper).

**GRAPHS IN DETAIL.**—The displacement of the *NPK-unit* from one sampling date to another for all treatments cannot be shown in one diagram, because of the confusion resulting from the intersection of the graphs from certain of the plots. The detailed graphs showing the course of nutrition are best presented by making use of three diagrams (figs. 5, 6, 7) so arranged that the graphs for the respective treatments are compared with the complete fertilizer plot.

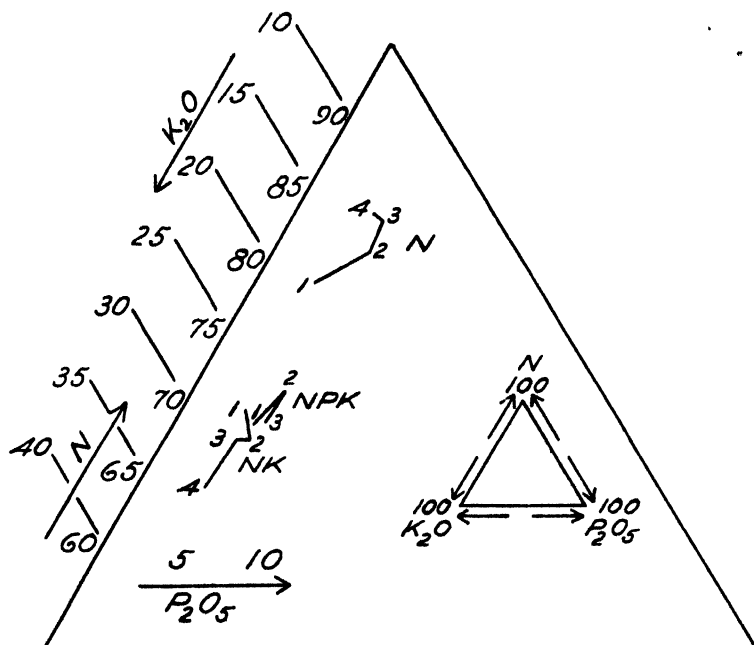


FIG. 6. Course of nutrition of plants growing on the N (no. 2), NK (no. 7), and NPK (no. 10) plots as indicated by the changes in their NPK-units from one sampling date to another.

*Foliar diagnosis of low-yielding group 3—N (no. 2), P (no. 3), and NP (no. 6).*—The *intensity of nutrition* is very low (range of the mean is 7.34 to 5.65). This low intensity is the result of the very low content of  $K_2O$  (see table II, column 3, for plots 2, 3, 6).

Nitrogen in the *NPK-unit* is very high (the range of the mean NPK-unit is 81.1 to 81.7). Potash is very low (the range of the mean NPK-unit is 13.9 to 9.56). Phosphoric acid is high in those which received phosphate additions, *viz.*, NP (no. 6) and P (no. 3).

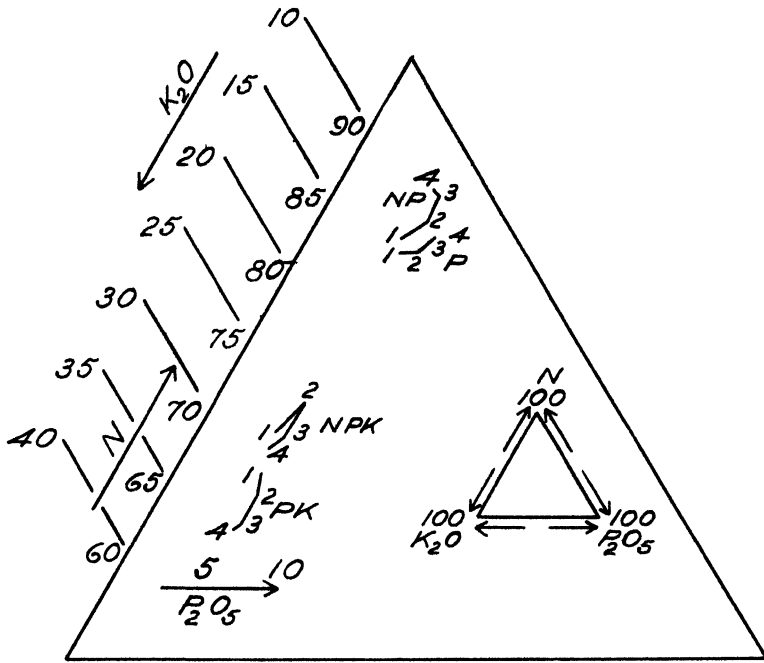


FIG. 7. Course of nutrition of plants growing on the P (no. 3), NP (no. 6), PK (no. 8), and NPK (no. 10) plots as indicated by the changes in their NPK-units from one sampling date to another.

*Foliar diagnosis of intermediate-yielding group 2—K (no. 4) and PK (no. 8).*—The mean intensity is 9.48 and 9.56, respectively, for the plants growing in the K plot (no. 4) and the PK plot (no. 8). In this group the intensity is slightly higher than that of the complete fertilizer plot (no. 10).

Nitrogen in the *NPK-unit* is much lower than in the low-yielding group 3 and also somewhat lower than in the higher-yielding group 1. The mean NPK-unit is 63.3 and 65.9 in PK (no. 8) and K (no. 4), respectively. Potash in the NPK-unit is higher than in both groups 1 and 3.

*Foliar diagnosis of high-yielding group 1—NK (no. 7) and NPK (no. 10).*—The mean intensity is 10.24 in NK (no. 7) and 9.39 in NPK (no. 10). The plants growing on plot NK (no. 7) have the highest intensity reported in this paper, but plants growing on three other plots in this tier (not reported in this paper) have still higher intensities. This high intensity of nutrition in NK (no. 7) relative to plants growing on the other plots is due to the phenomenon of the reciprocal effects of the elements (40). In this particular case the omission of phosphorus from the complete fertilizer has resulted in a much greater uptake of the elements remaining (compare columns 1, 2, 3 of table II for NK (no. 7) and NPK (no. 10)).

Nitrogen in the *NPK-unit* is much lower and potash much higher than

in the low-yielding group 3. Phosphoric acid in the NPK-unit has the lowest value in NK (no. 7) of any plot.

*Course (nature) of nutrition with respect to NPK-unit in group 3—N (no. 2), P (no. 3), and NP (no. 6).*—The course of the nutrition (*mode d'alimentation*) in this low-yielding group is strongly contrasted. This group, however, has one characteristic in common, *viz.*, nitrogen in the *NPK-unit* continuously increases at the expense of the potash with the increasing age of the leaves, as shown by the upward displacement of the graphs at each successive sampling period. In N (no. 2) and NP (no. 6) this tendency is quite marked. Consequently, the application of nitrogen without potash has had a strong tendency to reverse the direction of the nitrogen nutrition which the medium (soil) has tended to impose. This tendency is also observed but to a smaller extent with phosphate applied alone (P plot, no. 3), *i.e.*, without mineral nitrogen applications.

It is also to be noted that in the leaves of plants growing on the N plot (no. 2) and which, therefore, has received no phosphate additions for nearly 20 years other than that added in the system of green manuring, the phosphorus in the *NPK-unit* increases with the age of the leaf up to the third sampling, after which a decrease occurs. The proportion of phosphorus in the *NPK-unit* of the N (no. 2) plot is not, however, as high as that in the leaves from plants growing on the NP (no. 6) or P (no. 3) plots which received mineral phosphates. The continual accumulation of phosphorus throughout the whole period in the *NPK-unit* of plants growing on Plot P (no. 3) is very marked, signifying poor assimilation of this entity (element) by the plants growing on this plot.

*Course of nutrition with respect to NPK-unit in group 2—K (no. 1) and PK (no. 8).*—The graphs indicate that the course of the nutrition with respect to the *NPK-unit* of the plants growing on these plots is very different throughout the whole period. The relative yields of tubers from plot K (no. 4) to that of plot PK (no. 8) on this tier 1 is 95.1:90.8, associated with which is the fact that nitrogen in the *NPK-unit* of plants growing on plot K (no. 4) is higher than are those of plot PK (no. 8) throughout the whole period. The omission of phosphorus, therefore, has on this particular tier resulted in increasing the nitrogen in the *NPK-unit* of plants growing on plot K (no. 4) from one (PK, no. 8) classed as too low in nitrogen. This is another example of the phenomenon of reciprocal effects (40).

*Course of nutrition with respect to NPK-unit in group 1—NK (no. 7) and NPK (no. 10).*—The omission of mineral phosphates has in this case also changed the character of the nutrition with respect to the *NPK-unit*. On July 7 the proportion of nitrogen in the *NPK-unit* in the leaves of plants growing on plot NK (no. 7) is greater than in those from the complete fertilizer plot (no. 10). But, whereas in the former (plot no. 7) the nitrogen in

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# UREASE DISTRIBUTION IN *CANAVALIA ENSIFORMIS*<sup>1</sup>

SAM GRANICK<sup>2</sup>  
(WITH TEN FIGURES)

## Introduction

In this account the distribution of urease, as studied with the histological and quantitative methods which were described in the first paper of this series (2), will be discussed under the subheadings of the various plant structures. The definition of some of the terms used are as follows:

*Urease activity* is expressed in urease units. A urease unit (U.U.) is a quantity of urease which will produce 1 mg. of ammonia per minute when acting upon urea under the experimental conditions employed (2). The indicator method is not as sensitive as the quantitative method, and tissues containing less than 0.02 U.U. per gram of fresh weight are reported as negative with this method. Tissues containing less than 0.0001 U.U. are reported as negative with the quantitative method.

The *hypocotyl* is that part of the axis between the cotyledonary node and the external collet.

The *external collet* is the region below the hypocotyl, bearing a considerable tuft of lateral roots.

The *first internode* is the hypocotyl.<sup>3</sup>

The *first node* is the cotyledonary node.

The *second node* is that part of the stem axis from which the first foliage leaves arise.

*Canavalia ensiformis* or jack bean is an annual legume usually bushy and erect, 1 to 2 meters high; the tips of the branches inclined to twine; peduncles stout, 10 to 20 flowered; pods linear, 25 to 30 cm. long; seeds ellipsoid, shiny white, 22 × 14 × 8 mm. The plants grown in the greenhouse at Ann Arbor were 3 to 4 meters high and spindling. The distinctive taxonomical description of *Canavalia ensiformis* is given by PIPER (5). The gross morphology of the plant closely resembles that of *Phaseolus vulgaris* whose anatomy has recently been studied by DOUTT (1).

<sup>1</sup> Second of a series of three papers on urease distribution in *Canavalia ensiformis* and *Soja max.* The reader is referred to the thesis available at the University of Michigan library for more extensive data on urease in the jack bean, for descriptions of the relevant anatomical and embryological features of the plant, and for the literature on the presence of urease in various species of the plant kingdom.

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<sup>3</sup> This convenient designation, used solely for the purpose of this paper, is not technically correct according to morphological terminology which would consider the first internode as the region between the cotyledonary node and the first foliage leaves.



The bulk of the seed of jack bean consists of cotyledons which are made up largely of parenchyma cells with thickened hemicellulose walls possessing numerous tiny pits. SUMNER (6) estimates that the bean contains 0.12 to 0.06 per cent. urease and considers the crystalline urease to be globulin, because it is insoluble in the absence of neutral salts in the vicinity of its isoelectric point. About 25 per cent. of the air-dried seed is protein (4).

## Experimentation

### COTYLEDONS

Employing the histological urease methods, examination has shown that the cotyledons contain more urease than any other tissues of the plant. Studies of the seeds after two days of germination in sphagnum revealed that the enzyme concentration was greatest in the subepidermal cells, and especially in the bundle sheaths, and phloem tissue, that is, in those cells of the cotyledons containing the densest cytoplasm (fig. 1). The bundles of the cotyledons immediately adjacent to and merging with the conducting bundles of the hypocotyl appeared to contain less urease than the other bundles of the cotyledons. This may be due to a more rapid elongation of the bundles adjacent to the cotyledons, the enzyme thus becoming more diffuse, or it may be due to decomposition of the enzyme in the bundles. As germination proceeds, urease decreases rapidly in the epidermis and in the two to three layers of subepidermal parenchyma cells. The enzyme disappears from some parenchyma cells more rapidly than from others. No relation could be noted between the enzyme content of the cells close to the bundles, and those more or less isolated from the bundles. This suggests that there is no transport of active urease to the bundles. The cotyledons which fell off after 20 days still contained relatively large quantities of urease. Sectioning at this stage revealed that the cells were thin-walled with large vacuoles, and contained numerous chloroplasts but little starch.

TABLE I  
UREASE CONTENT OF COTYLEDONS OF JACK BEAN SEEDLINGS

DAYS AFTER PLANTING	FRESH WT. PER COTYLEDON	DRY WT. PER COTYLEDON	U.U. PER GM. FRESH WT.	U.U. PER COTYLEDON
<i>days</i>	<i>gm.</i>	<i>gm.</i>		
0 .....	0.651	0.560		
1 .....	1.05	0.561	31.2	32.8
3 .....	1.25	0.532	25.9	32.5
5 .....	1.15	0.456	24.4	28.0
7 .....	1.26	0.377	12.6	15.9
10 .....	1.17	.....	11.5	13.5
20* .....	0.483	.....	1.08	0.523

\* The cotyledons had just fallen off; they were quite thick, yet flaccid.

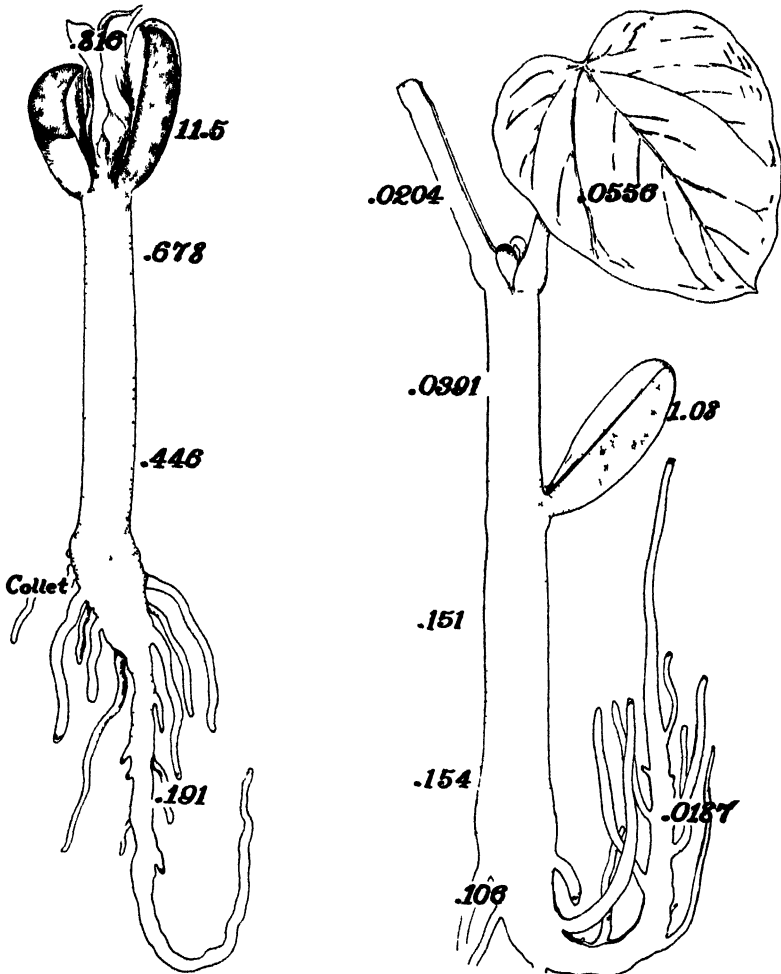


FIG. 1. Composite picture of urease distribution in the various structures of jack bean seedlings 10 and 20 days old. The figures opposite the various regions of the seedling represent the number of urease units per gram of fresh weight of the region, as determined by the quantitative method. Stippling indicates the relative urease concentration as revealed by the histological methods.

Quantitative studies were made of the urease content of cotyledons during the development of the seedlings. It will be noted from table I that the urease content of the cotyledon decreases rapidly as the seedling becomes older. The data are interpreted as indicating, not that the enzyme is gradually inactivated by poisons or other inactivators, nor that it is transported intact to other tissues, but that it is broken down just as are other storage proteins of these cells, into some soluble compounds which are then

transported to other plant parts. This viewpoint will be discussed later in more detail.

#### RADICLE

In the radicle 1 cm. long (fig 2.) the histological reagent shows urease to be present in all of its parts except the primary xylem elements. The

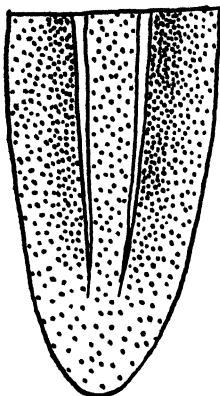


Fig. 2. Urease concentration in a radicle 1 cm. long.

inner cortical cells contain the most enzyme, the outer cortical cells and pith less enzyme, and the cells of the radicle tip still less. The dermatogen appears to contain little or no enzyme; it is, however, slightly more acid (pH 4.4) than the other tissues. The weak indicator-change in the dermatogen may possibly be due to the fact that more acid has to be neutralized before the indicator shows an alkaline reaction than in the other tissues. Therefore, a positive statement cannot be made concerning its relative urease content.

Cross-sections of a 3-cm. radicle reveal a decrease of urease in the region in which cell elongation has occurred, that is, at approximately 2 cm. distal to the tip. At this level there is less enzyme in the pith than in the cortex. After four days' germination the radicle has attained a length of 5 cm. and the collet region with its tuft of secondary roots has appeared.

#### COLLET

The collet region was made the subject of particular study, since it is in this region that the various stages of lateral root differentiation can most readily be obtained. In 5-day-old seedlings urease tests showed that the enzyme was most concentrated in the elongated narrow cells of the outer cortex (pH 4.0); the inner cortex and pith (pH 5.5) contained slightly less enzyme. It was difficult to judge the urease content in lateral root primordia because of the relatively high enzyme activity of the parenchyma cells of

the collet. The lateral root primordia arising endogenously from the pericycle cells opposite the protoxylem always contained less urease than did the parenchyma cells. By removing the larger lateral roots (1 to 3 cm.) and examining them separately, it was found that they contained very little enzyme except in the apical meristems. In 4-week-old seedlings, sections of the collet indicated a low urease activity, the pith containing somewhat more urease than the cortex. In 7-week-old plants the pith gave a barely perceptible test for urease; the cortex gave no test.

### Root

In the 5-cm. radicle, a distinction between root and hypocotyl is already evident, owing to the appearance of a lateral tuft of roots at the collet region. An examination was made of the urease content at different levels of the primary root. The enzyme was present in the apical meristem. It was absent in the cambium and its derivatives of secondary phloem and xylem which were already differentiated in the region 2 cm. above the tip. The



FIG. 3. Urease concentration and distribution in meristems of jack bean (as shown by intensity of stippling in camera lucida drawings).

I and II, sections made through different levels of the same pedicellar gland, showing urease in the very young flower meristems and in the gland.

III, IV, VIII, IX, urease distribution in later stages of flower differentiation.

V, distribution of urease in a longitudinal section of root tip.

VI, urease content of a cluster of promeristems of a leaf axil.

VII, distribution of urease in an apical vegetative meristem.

parenchyma cells of the cortex and pith, however, contained urease throughout the entire length of the root.

Seven days after planting, the root had increased to 20 cm. At this stage, the root tip contained very small quantities of urease. Roots, sectioned longitudinally in paraffin or on a freezing microtome, showed a positive reaction for urease in only about 5 per cent. of the root tips examined. The enzyme in these root tips was strictly delimited to the meristematic region (fig. 3, V). Whole root tips also were examined. The following modified procedure gave good results. A number of root tips were placed on a slide and treated with haematoxylin, in 20 per cent. alcohol, for 15 minutes. The dye penetrated into the cells and the root tips became yellow. (A few root tips, which appeared to be injured or sickly, turned red, that is, they were more alkaline than the rest. These were discarded.) Excess dye was drained off, 1 per cent. urea solution was added, and a cover slip applied. After 2 to 10 minutes, a red color appeared which varied from a very weak positive to a weak positive test. Every root showed this red region at the meristematic zone, and not far above it. The variations in urease distribution in the root tips examined by this method are shown in figure 4. The maximum urease

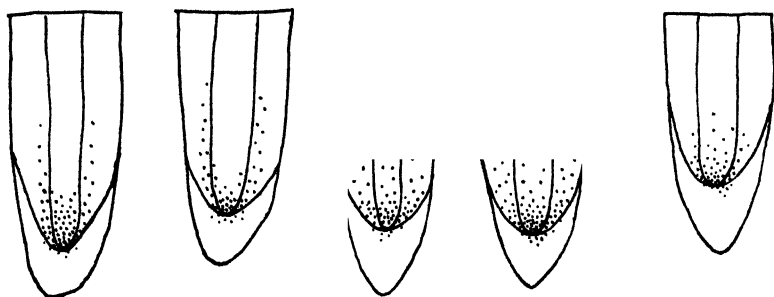


FIG. 4. Urease distribution in root tips.

content is in the region of actively dividing cells. In some root tips there is more urease in the periblem than in the plerome, and in others the opposite is true.

A large number of root tips from 13-week-old plants were examined. Most of these root tips gave a negative reaction for urease with the staining reagents. However, those that were positive showed that urease was located only in the meristematic region.

The studies of LINDERSTRØM-LANG and HOLTER (3), on the quantitative distribution of peptidase in the germinating barley root, led them to conclude that the maximum peptidase activity was related to the region of cell elongation and not to the region of rapid cell division. The experiments on urease relate the maximum urease concentration to the region of rapid cell division.

The data for the quantitative urease determinations on the roots of the jack bean are given in table II. It is seen from these figures that the urease

TABLE II  
UREASE CONTENT OF ROOTS OF JACK BEAN SEEDLINGS

DAYS AFTER PLANTING	STRUCTURE	FRESH WT.	U.U. PER GM. FRESH WT.		U.U. PER UNIT STRUCTURE	
			+ COLLET	- COLLET	+ COLLET	- COLLET
<i>days</i>		<i>gm.</i>				
1	radicle .....	0.025	19.2	.. .. .	0.480	.....
3	radicle .....	0.300	2.94	.. .. .	0.824	.....
5	root + collet .....	0.310	0.434	.....	0.134	.....
7	root + collet .....	0.788	0.169	.....	0.133	.....
10	root + collet .....	0.645	0.191	.. .. .	0.123	.....
20	root .....	0.625	.. .. .	0.0187	.....	0.0117
..	root + collet .....	0.997	0.0515	.....	0.0514	.....
28	root .....	2.37	.. .. .	0.0114	.....	0.0271
40	root .....	3.31	.. .. .	0.0079	.....	0.0263
.....	root + collet .....	3.77	0.0105	.....	0.0396	.....

activity per gram of fresh weight decreases with increasing age of the roots. This is to be expected if one assumes: that (a) the cells of the root, back of the region of cell division, do not possess any marked ability to synthesize or elaborate urease, in which case only a dilution of the enzyme already present in the cell would occur during cell elongation; that (b) owing to the partial sloughing off of the outer cortical cells of the root, and a partial disintegration of the pith, these urease-containing cells are destroyed and the enzyme in them is likewise destroyed; that (c) owing to the increase of dead xylem cells, the percentage of living cells in the root decreases and therefore the urease activity per unit of fresh weight also decreases. From the figures on the urease activity per unit tissue of "root + collet," it is seen that a definite decrease of urease must occur even in the early stages of root formation. This decrease of the enzyme is not readily evident during the first 10 days after germination because of the rapid increase in the number of new roots, and consequently of young cells containing urease. After this time, the number of roots increases slowly and the value for total urease drops off rapidly. The data also indicate that during the first 10 days the collet region possesses approximately five times as much urease activity as the roots.

#### HYPOCOTYL

The hypocotyl of the 5-day-old seedling possesses a high urease content throughout its entire length of 4 cm. The enzyme appears mainly in the parenchyma cells of the cortex and pith. The epidermis and the small sub-epidermal parenchyma cells contain little of the enzyme. No urease could be detected, with the histological reagents, in the cambium or its derivatives.

The decrease of urease activity follows the elongation of the hypocotyl as would be expected if dilution of the enzyme were taking place. The stippling in figures 1 and 5 indicates the relative urease activity of the 10-, 20-, 28-, and 40-day-old seedlings. The enzyme does not continue to decrease indefinitely, for in the 12-week-old plant the hypocotyl still gives a weak reaction in the pith and cortex with the histological reagent. The content of urease in the hypocotyl is always highest just below the cotyledons and just above the collet region, disappearing most slowly from the collet region. Whether the cells in these regions remain small, or whether less urease is catabolized, has not been determined.

From the quantitative data in table III, it may be observed that the change in urease activity is quite marked, decreasing from 0.777 U.U. per

TABLE III  
UREASE CONTENT OF HYPOCOTYL (FIRST INTERNODE)

DAYS AFTER PLANTING	STRUCTURE	FRESH WT.	U.U. PER GM. FRESH WT.	U.U. PER UNIT STRUCTURE	
				ENTIRE HYPOCOTYL	PARTS OF HYPOCOTYL
<i>days</i>		<i>gm.</i>			
1	Radicle . . . . .	0.025	19.2	0.480	.....
3	Radicle . . . . .	0.300	2.94	0.824	.....
5	Hypocotyl 3.5 cm. . . . .	0.480	1.62	0.777	.....
10	Hypocotyl 7 cm. . . . .	0.921	0.561	0.518	
	Upper half . . . . .	0.460	0.678		0.312
	Lower half . . . . .	0.461	0.446		0.206
20	Hypocotyl 12 cm. . . . .	1.802	0.153	0.275	
	Upper half . . . . .	0.857	0.151	.....	0.129
	Lower half . . . . .	0.945	0.154	..	0.148
28	Hypocotyl . . . . .	1.401	0.0550	0.0770	.....
40	Hypocotyl . . . . .	1.708	0.0317	0.0541	.....

hypocotyl in the 5-day-old seedling to 0.0541 U.U. in the 40-day-old plant. The changes in structure of the hypocotyl with age, such as the conversion of some parenchyma cells into pericyclic and collenchymatous tissue, etc., do not account for the marked decrease in total urease content of the hypocotyl. A decrease in the enzyme content of the parenchyma cells is evident.

### STEM

The apex of the stem was made the subject of a detailed study since this material contains, within the short space of 3 mm., the embryonic meristem and the differentiating tissues. Longitudinal sections, cut at 35 and 60  $\mu$  were tested with the urease reagents. These showed that urease was located in greatest quantity in the primordial meristem. With the enlargement of the isodiametric cells of the meristem to form cortex and pith, the enzyme rapidly decreased in concentration, being absent in some stem tips at the region where metaxylem elements are already visible.

A few photomicrographs (fig. 6) were taken of sections of apical buds treated with the haematoxylin-nickel reagent for urease. In the presence of ammonia, a dark purple lake is formed which shows up black in the photographs. The photographs do not indicate the actual sharpness with which the enzyme may be localized by this method, since it was necessary to permit the development of a more intense and therefore a somewhat more diffuse color in order properly to photograph the sections. Figure 6 A shows two meristems containing much urease, while figure 6 B shows two meristems one of which contains more enzyme than the other.

There were great variations in the enzyme content of the various apical and axillary buds examined. Sections of some buds gave no urease test. Most of them gave only a weak reaction (fig. 3, VII). A few gave fairly strong reactions. The weakly reacting sections evidenced limitation of urease to the promeristem of the tip and to the promeristems in the leaf axils. With sections of higher urease activity both the promeristems and the procambium possessed a relatively high urease content; furthermore, the cortex, the pith, or both together, gave a positive urease reaction extending well into the elongation-region (3 mm. from the tip). The procambium strands gave weaker reactions as they differentiated, and by the time a few metaxylem elements had appeared they gave no reaction for urease. In these buds it was difficult to judge the urease content of the cambium or its derivatives because of the urease activity of the neighboring parenchyma cells. In cross-sections of young stem tissue 15 mm. below the apical bud, the enzyme was just faintly detectable only in the pith.

A comparison was made between the urease activity of the axillary and the apical buds taken from the same plant. It was found that some axillary buds contained more urease than the apical buds, or *vice versa*. It also appeared that apical buds of vigorously growing shoots possessed a somewhat higher urease activity than apical buds of slower-growing shoots.

The quantitative data on the urease content of the stem internodes (table IV) indicate that the U.U. per gram of fresh weight values are highest in the uppermost, and therefore the youngest, internodes. This is to be expected

TABLE IV  
UREASE CONTENT OF DIFFERENT INTERNODES OF THE STEM

INTERNODE	AVERAGE LENGTH	FRESH WT.	U.U. PER GM. FRESH WT.	U.U. PER INTERNODE
	<i>cm.</i>	<i>gm.</i>		
6	3 (leaf bud)	0.0088	0.066	0.0006
5	5	0.150	0.0127	0.0019
4	10	0.585	0.0059	0.0034
3	9	0.644	0.0049	0.0028



since the histological studies on the stem tip showed that the youngest cells possessed the highest urease activity. The urease activity per gram of fresh weight in the lower internodes decreases progressively with age owing to the dilution of the enzyme and probably also to a catabolism of the enzyme.

### EPICOTYL

The region above the cotyledonary node, *i.e.*, the epicotyl, is considered separately from the other internodes because it is one of the organs which is already developed in the seed and these organs have been found to possess very high urease contents. Sections of the young, second internode show

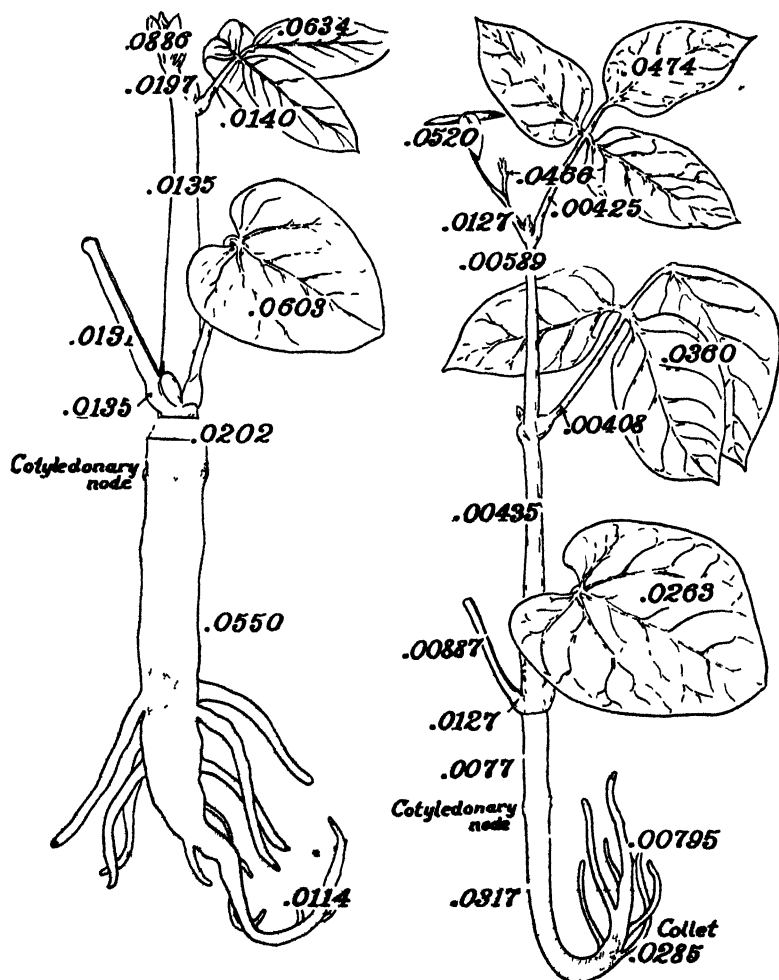


FIG. 5. Composite picture of urease distribution in the various structures of the jack bean seedlings 28 and 40 days old. (See legend of fig. 1.)

active urease in the pith and cortex. As the internode increases in length, the enzyme concentration decreases until after 28 days (fig. 5) it is detectable only close to the cotyledonary node. From the quantitative data in table V it may be seen that the urease content per epicotyl is highest when the plant is 15 days old. The most rapid elongation has occurred between the tenth and fifteenth day. After this period of rapid elongation the urease

TABLE V  
UREASE CONTENT OF EPICOTYL (SECOND INTERNODE)

AGE OF PLANT	LENGTH	FRESH WT.	U.U. PER GM. FRESH WT.	U.U. PER EPICOTYL
<i>days</i>	<i>cm.</i>	<i>gm.</i>		
3	0.9 (plumule)	0.0120	1.35	0.0162
15	3.5	0.180	0.222	0.0401
20	5.0	0.412	0.0391	0.0161
28	5.5	0.512	0.0202	0.0103
40	5.2	0.675	0.0077	0.0052

content per epicotyl drops off markedly. Urease activity per gram of fresh weight is greatest in the meristematic tissue of the young 9-mm. plumule and decreases rapidly as these cells elongate and age. This same sequence of urease changes with age has also been found in the other stem internodes.

#### LEAVES

Examination of longitudinal sections of the differentiating leaves of a large number of axillary and apical buds revealed that the very young leaves enveloping the growing point contain urease, at times more, and at other times less than the growing point itself. The enzyme content of the buds as a whole varied greatly, some containing much, others little urease. No reason has as yet been found for this variation. The procambial tissue at the base of the developing leaf gave a strong reaction for urease. Even at a slightly later stage, when a portion of the procambium strands had differentiated into definite protoxylem elements, the content of urease was still high in the protophloem and procambium. No differences in urease activity were noted among the various parenchyma cells.

In a study of leaflets 10 mm. long it was found that the veins gave a stronger urease reaction than did the other portions of the leaf blade. Examination of the veins indicated that the enzyme was limited to the large area of parenchyma cells below the vascular bundles, and to a lesser degree to the bundle sheath, the reaction of the phloem being doubtful.

Sections of leaflets 4 cm. long from the first leaf below the apical bud again showed that the cortical cells of the midrib and of the other main veins contained more urease than did the other cells of the leaf blade. The strong-

est urease reaction of the leaf was given by the cortical cells of the midrib at the base of the leaf. No difference could be noted in the urease content of parenchyma cells throughout the length of the leaf. In cross-sections, the enzyme appeared to be more concentrated in the palisade cells than in the

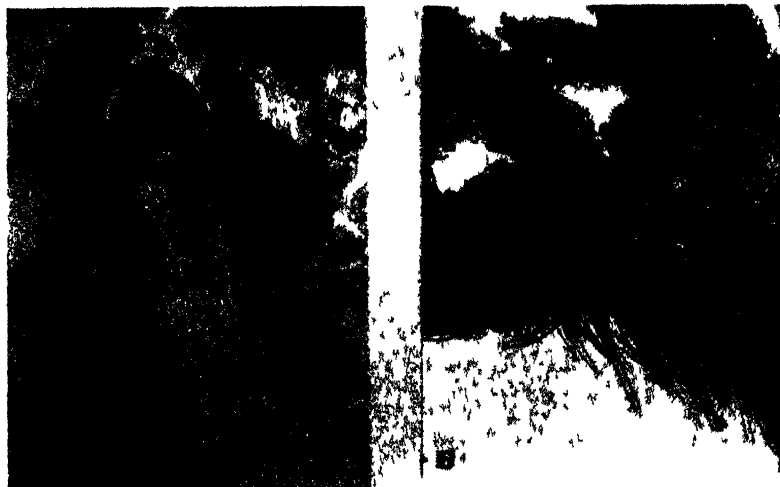


FIG. 6. Urease distribution in meristems of apical buds of jack bean. Darker areas indicate higher urease content.

spongy mesophyll. This may be due, however, to the compactness of the palisade cells which, under the conditions of the test, would effect a more rapid increase in alkalinity than would the loose mesophyll cells, even though the urease contents were the same, cell for cell. The epidermal cells appeared to contain less urease than the mesophyll cells. In leaflets 12 cm. long, no indications of the enzyme distribution could be obtained because of the low urease content.

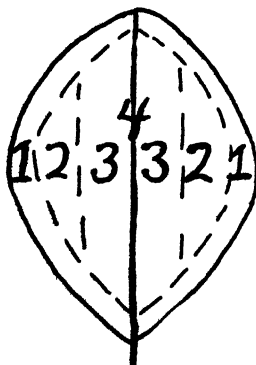


FIG. 7. Leaflet showing place from which samples were taken for urease determinations.

Quantitative determinations were made on the distribution of urease in different portions of leaflets 12 cm. long and 5.3 cm. wide. Portions of these nearly mature leaflets were taken as shown in figure 7. It is seen from table VI that the data on U.U. per gram of fresh weight indicate that urease

TABLE VI  
UREASE CONTENT OF PORTIONS OF LEAFLET (FIG. 4)

No.	PART OF LEAFLET	FRESH WT.	U.U. PER GM. FRESH WT.
		<i>gm.</i>	
1	Outermost 5-mm. edge .....	0.720	0.0310
2	10-mm. middle strip .....	0.998	0.0345
3	10-mm. inner strip .....	1.161	0.0342
4	Midrib .....	0.365	0.0123

activity is about the same throughout the various portions of the lamina. The enzyme content of the outermost edge of the lamina is somewhat less than that of the other portions of the lamina. This may or may not be due to the fewer parenchyma cells as compared to epidermal cells in this portion of the lamina. The midrib of the leaflet contains only about one-third as much urease per gram of fresh weight as the other parts. As has been mentioned above, the parenchyma cells in the veins contain much urease. The relatively low figure for U.U. per gram of fresh weight in the midrib is due to the large proportion of primary xylem and of collenchyma cells which contain little or no urease.

A quantitative study was made on the amounts of enzyme in leaves of different sizes. Under similar environmental conditions the young, actively growing ternate leaves of the same size showed remarkably similar values for urease content. Apparently the position of the leaf on the stem makes little difference. Indeed, it was possible to predict approximately the total urease content of any size of actively growing leaf because of this constancy of relation. From table VII it is evident that the youngest tissues possess the highest urease activity per gram of fresh weight. As the leaves grow older, these values decline. The figures for "U.U. per unit structure" show clearly that a definite synthesis of urease has taken place during the expansion of the cells, and long after cell division has ceased in the young leaf. Contrasting the leaf 6.5 cm. long and the fully expanded leaf 15 cm. long, it is seen that the urease content has increased from 0.037 to 0.149 U.U. per leaf.

#### PLUMULE LEAVES

The first leaves (plumule leaves) to appear after the germination of the seed are a pair of simple, opposite, cordate leaves located at the second node. These were laid down in their embryonic condition within the developing

seed. As noted previously, the tissues formed in the developing seed possess, in their early stages, a higher urease activity than those which develop from the meristems after germination. Because of this higher content, detailed studies of urease distribution in these leaves could be made with the histological reagents.

TABLE VII  
UREASE CONTENT OF DEVELOPING TERNATE LEAVES

STRUCTURE	FRESH WT.	U.U. PER GM. FRESH WT.	U.U. PER UNIT STRUCTURE
	<i>gm.</i>		
1-mm. apical bud .....	0.0016	0.08	0.0001
3-mm. apical bud .....	0.0028	0.05	0.0001
17-mm. apical bud at 6th node .....	0.0265	0.046	0.0012
3.5-mm. leaf .....	0.0088	0.066	0.0006
1.4-cm. leaf at 5th node .....	0.0385	0.053	0.0020
6.5-cm. leaf blade at 4th node .....	0.780	0.047	0.0370
15.0-cm. leaf blade at 3d node .....	4.115	0.036	0.149
Petiole of leaf at 4th node .....	0.603	0.0042	0.0026
Petiole of leaf at 3d node .....	0.579	0.0041	0.0024

Sections of the young plumule leaf (3 mm. long) showed a strong urease reaction throughout the leaf, the veins containing slightly less enzyme than the embryonic parenchyma cells. In the slightly older leaf (8 mm. long) the large cortical cells of the midrib contained less urease than did the embryonic parenchyma and epidermal cells. In the leaves 20 mm. long, the picture of urease distribution was similar to that obtained with the trifoliate leaves, that is, the palisade cells gave the strongest urease reaction, the mesophyll cells less, and the epidermal cells still less. Epidermal layers, stripped from the leaves and examined for urease, gave indications of the presence of only traces of enzyme. Sections through a leaf 8 cm. long from a 17-day-old plant showed that the greatest urease concentration was present in the parenchyma cells of the midrib lying below the vascular bundles. The urease reaction was especially strong at the base of the leaf, *i.e.*, in the secondary pulvinus. In the petiole proper, only a slight urease activity was noted. At the base of the petiole, in the pulvinus, only the lower portion of the cortex contained the enzyme (fig. 5).

Determinations were also made of the quantity of urease in the plumule leaves at various stages of their development. The leaves follow the same trends of urease content as those noted for the stem internodes and the roots (table VIII). The youngest leaves have the highest urease activity per gram of fresh weight and as the leaves become older the urease activity per gram of fresh weight diminishes rapidly. The urease content per unit of organ shows that a definite synthesis of the enzyme takes place until elongation of

TABLE VIII  
UREASE CONTENT OF THE PAIR OF PLUMULE LEAVES

AGE OF PLANT	STRUCTURES	FRESH WT.	U.U. PER GM. FRESH WT.	U.U. PER UNIT STRUCTURE
<i>days</i>		<i>gm.</i>		
10	2-cm. leaves .....	0.0101	0.816	0.083
20	8.5-cm. leaf blades .....	2.066	0.0556	0.115
28	13-cm. leaf blades .....	4.990	0.0603	0.306
40	13-cm. leaf blades .....	5.730	0.0263	0.150
28	13-cm. leaf blades:			
	main veins .....	0.850	0.0377	0.032
	blade minus main veins .....	4.14	0.0662	0.274
20	Both petioles .....	0.538	0.0204	0.0110
28	Both petioles .....	0.662	0.0132	0.0088
40	Both petioles .....	0.783	0.0098	0.0078

the cells has practically ceased. From that time on inactivation or catabolism of urease occurs. The petioles undergo these changes more rapidly than do the leaves. The urease content of the plumule leaves in their adolescent stage (approximately 2 cm. long) is much higher (0.81 U.U. per gm. fresh weight) than that of the ternate leaves of similar size (0.05 U.U. per gm. fresh weight). Stipules of the second node were also examined, and, as would be expected, they undergo changes in urease content similar to the leaves.

#### FLOWER AND FRUIT

The flowers arise on knoblike protuberances, the pedicellar glands, which may be borne sessile in the axil of a leaf in close proximity to the axillary vegetative bud; or they may arise from a stalk, the peduncle, which may grow out to a length of 10 to 15 cm. and upon this peduncle may be borne one or more pedicellar glands, each of which may bear 3 to 5 flowers (fig. 3, IV, VIII).

The distribution of urease was studied in sections of flower primordia, in a manner similar to that described for the vegetative primordia. A photomicrograph of a longitudinal section, through a nodal region, stained with the lake reagent for urease, is shown in figure 8. It will be noted that there are several promeristems present in a very early stage of differentiation, all of which stain darker than the neighboring tissues, and thus contain more urease. It is probable that the cluster of three promeristems at the left are the "anlage" of the flower primordia and that the promeristem at the right will differentiate into a vegetative primordium. Another view of a cluster of promeristems in a leaf axil may be seen in figure 3, VI, where urease concentration is indicated by the intensity of stippling.

Urease distribution in later stages of flower differentiation is illustrated in figure 3. Figure 3, VIII shows a section through a pedicellar gland bear-



FIG. 8. Longitudinal section of axillary meristems showing the distribution of urease.

ing three flower primordia. Figure 3, I and II, are of sections made through different levels of the same pedicellar gland. They indicate that a relatively high urease activity extends from the flower promeristems into the regions of the pedicellar gland where the cells are still small and active. Later stages of flower differentiation are represented by figure 3, IV, III, and IX. From the numerous observations made on flower primordia, it may be said that, in general, urease is relatively highest in concentration in the flower promeristems; in the differentiating flower it is highest in the primordia of the pistil and the cells connecting the pistil with the pedicellar gland; it is less in the stamen primordia, and least in the primordia of the calyx and corolla.

No urease can be detected in the peduncles with the indicator reagents. A quantitative determination on peduncles varying from 1 to 9 cm. in length gave an average urease activity of 0.0165 U.U. per gram of fresh weight (table IX).

The pedicellar glands are made up of a spongy mass of parenchyma cells. Only those regions of the parenchyma which join or abut on the flower primordia give a faintly positive urease reaction with the histological re-

TABLE IX  
UREASE CONTENT OF FLOWER BUDS

STRUCTURES	FRESH WT.	U.U. PER GM. FRESH WT.	U.U. PER UNIT STRUCTURE
Peduncles (1-9 cm. long) .....	.....	0.0165	.....
Pedicellar glands .....	.....	0.0199	.....
Tip of raceme; 12 flower buds each less than 2 mm. (av. wt. 0.0064 gm.) .....	0.0765	0.0542	0.00034 (average)
5-mm. flower bud .....	0.0144	0.0241	0.00035
13-mm. flower bud .....	0.152	0.00745	0.00112
14-mm. flower bud (just opening)	0.210	0.00683	0.00143

agents. The total urease activity of these glands is relatively low (0.0199 U.U. per gram fresh weight). From the pedicellar glands arise the flower primordia which contain a fair amount of urease (0.0542 U.U. per gm. fresh weight). As growth and differentiation of these cells continue, urease activity decreases (table IX), until, in the flower bud which is just opening, it is down to 0.0068 U.U. per gram of fresh weight. Although the urease activity per gram of fresh weight has decreased, there has been a definite synthesis of urease per flower bud.

When the flower is mature the pollen grains possess a low but definite urease activity. The pistil at this stage also has a low urease activity (0.0028 U.U. per gm. fresh weight).

Since the endosperm contains 3n or 33 chromosomes, it was considered especially interesting to determine the urease activity of this tissue. Sections of a bean 10 mm. long treated with a urease reagent showed that urease, if present in the endosperm, was below that concentration which could be detected by the histological method. It was however found that the endosperm could be readily removed *in toto* from the embryo sac. A number of endosperms were therefore removed and placed in small test-tubes and urea and indicator added until the total volume was 0.20 cc. Appropriate controls were also run. The endosperm tissue was not crushed. After one hour a definite positive indication of the presence of urease in the endosperm was observed. It is estimated that the endosperm had an approximate urease activity of 0.005 U.U. per gram of fresh weight. This same procedure was used in determining the presence of urease in mature pollen grains.

In a seed, 10 mm. long, the radicle of the embryo is somewhat less than 1 mm. The radicle at this stage possesses a definite but low urease activity. The cotyledons, consisting of small thin-walled cells, also possess a low urease activity. The inner integument consists of two layers of parenchyma cells and an epidermal layer abutting on the embryo sac. This integument has the highest urease activity of any of the tissues in a seed 10 mm. long, the urease activity being somewhat greater at the chalazal end.



The quantitative data (table X) on the urease content of the maturing bean reveal a steady increase of urease as the bean develops. In the young bean the weight of cotyledon and endosperm is negligible compared to that of the testas, and the values for U.U. per gram of fresh weight and for U.U. per bean up to the stage where the bean is 13 mm. long actually indicate the changes occurring in the testas. The testas pass through the same stages as other structures previously described. There is a decrease of U.U. per gram of fresh weight as the cells of the testa enlarge and mature; however, the urease activity per testa increases until the bean is approximately 15 mm. long and thenceforth decreases as the bean continues to enlarge.

TABLE X  
UREASE CONTENT OF THE MATURING BEAN

LENGTH OF BEAN	FRESH WT.	U.U. PER GM. FRESH WT.	U.U. PER UNIT STRUCTURE
	<i>gm.</i>		
4- 6-mm. bean .....	0.0117	0.0822	0.00096
7- 9-mm. bean .....	0.0575	0.0467	0.0027
11-13-mm. bean .....	0.185	0.0262	0.0048
17-mm. bean .....	0.648	.....	0.178
Embryo .....	0.352	0.495	0.174
Testa .....	0.296	0.0162	0.0048
20-mm. bean .....	1.080	.....	0.400
Embryo .....	0.646	0.615	0.397
Testa .....	0.434	0.0080	0.0035
22-mm. bean minus testa .....	1.60	.....	2.65
Cotyledons .....	1.58	1.65	2.61
Radicle and plumule .....	0.0162	2.75	0.044

As the cells of the cotyledons continue to divide and enlarge in the developing embryo, storage of proteins, starch, and hemicelluloses proceeds vigorously and the urease activity likewise increases very rapidly until the bean has attained its full size. In the radicle and plumule the cells elongate only slightly; there is less storage of carbohydrates than in the cotyledons but the cells accumulate proteins and also increase rapidly in urease activity. From the data in table X it is seen that the urease activity of the radicle and plumule of the mature bean (2.75 U.U. per gm. fresh weight) is even higher than the urease activity of the cotyledons (1.65 U.U. per gm. fresh weight). This dominant synthesis of proteins together with urease in meristematic cells of the embryo has already been noted for the other meristematic tissues of the plant with the exception of the cambium, where no detectable amount of urease is produced.

#### ENTIRE PLANT

A survey of the changes in the urease content of the jack bean plant as a whole, throughout its life cycle, is given in the graph of figure 9. Total

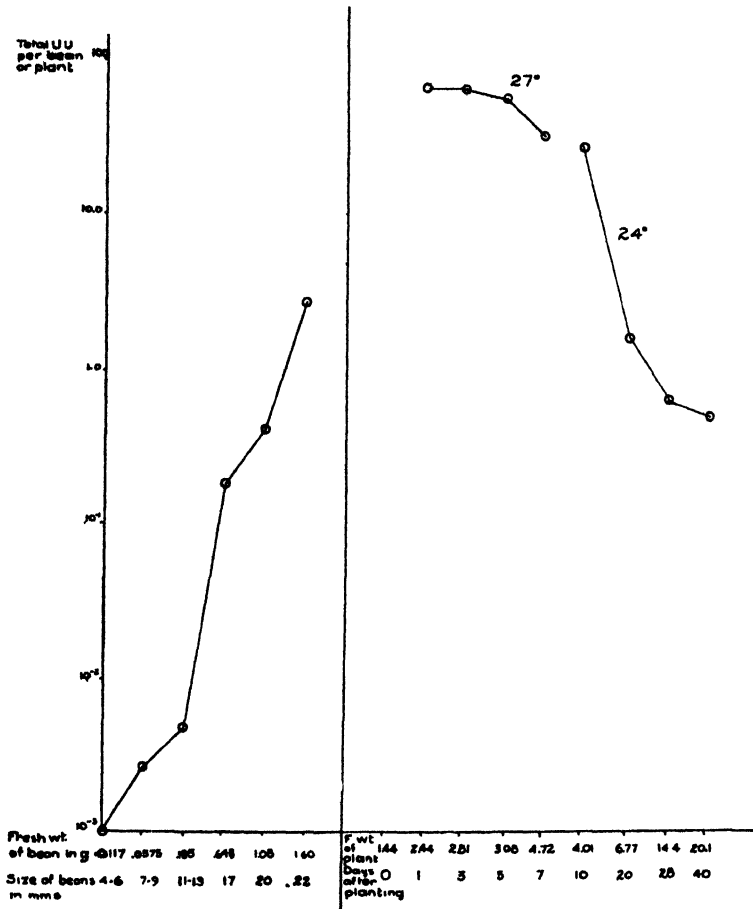


FIG. 9. Graph representing the total urease content of young maturing seeds and of seedlings at various ages after planting. "Total U.U. per bean or plant" is plotted logarithmically along the axis of ordinates. Various stages of seed or plant development are plotted on the axis of abscissas.

urease is plotted logarithmically on the ordinate, and the various stages of development are indicated on the abscissa. As the beans develop and mature in the pods, a rapid synthesis of urease takes place, the urease values increasing over 1000 times when a comparison is made between the urease content of the very young bean (4-6 mm. long) and the nearly mature bean (22 mm. long). The stages of complete maturity and drying were not determined in this plant.

Upon germination the seedlings develop rapidly. Thermograph records were kept and the average temperatures noted at which urease in the seedlings decreases with age, changing from 66 U.U. per seedling after one day's ger-

mination to 0.45 U.U. in the 40-day-old plant. The most rapid decrease in urease content occurred in the 10- to 20-day-old plant. In the 20-day-old plant the cotyledons were about to fall off. Although total urease determinations were not carried out on plants over 40 days of age, it may be said from other data that there will be a slight increase in urease content when more leaves have developed on the plant. Still later, when the seeds begin to form in the pods, a marked increase will take place.

When the data on the urease content of the other plant structures are considered, it becomes evident that figure 9 represents, to a large degree, the urease changes of the cotyledons. The cotyledons contain such preponderating quantities of urease as to overshadow any changes of urease content occurring in the other plant structures. Even when the cotyledons are about to fall off in the 20-day-old plant, they still contain about two-thirds of the total urease of the plant. The marked decrease in urease in the 10- to 20-day-old plant is due to the marked decrease in urease content of the cotyledons. It is interesting to note that even after the cotyledons have fallen off, the urease content per plant still continues to decrease. The decrease in the total amount of urease in the plant is therefore not wholly due to the decrease in the cotyledons.

The distribution of the enzyme in the various tissues of seedlings 10, 20, 28, and 40 days old is illustrated in composite pictures in figures 1 and 5. The stippling in these figures indicates the relative urease concentrations as revealed by histological methods. The figures opposite each structure express the urease units per gram of fresh weight as determined by the quantitative method. A description of the specific urease changes of the individual plant structures will be found under their respective headings in the previous pages.

### Discussion

It was realized, only after the analyses were nearing completion, that the urease content in different plant structures, as the internodes, leaves, roots etc., follow similar trends which are related to the ages of these structures. The curves in figure 10, in which the relative ages of the structures are plotted along the abscissa, indicate these general trends. The urease activity per gram of fresh weight (fig. 10 A) is highest when the cells of the young structure are actively dividing, and decreases as the cell of the primary tissues<sup>4</sup> grow older. The total urease content of a plant region (fig. 10 B) increases rapidly during the period of cell division of the primary tissues, and more slowly during the period of cell elongation until, at about the time

<sup>4</sup> The term *primary tissues*, as here used, has reference to those tissues developing from the apical meristems in contrast to the *secondary tissues* which develop from the cambium.

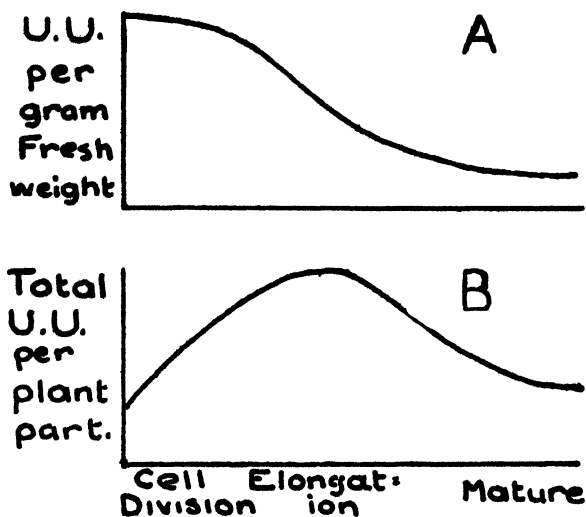


FIG. 10. Changes in urease activity in relation to physiological age.

primary growth has ceased, the plant part contains a maximum urease content.

Consideration of the histology of the seedlings makes it evident that the preponderating cells are the parenchyma cells. These cells originate by the elongation of the isodiametric cells of the apical meristems and appear to be the least differentiated of the cells derived from the meristems. Since it has been found, with the indicator reagents, that neither the cambium nor its derivatives contain perceptible quantities of urease, it becomes apparent that the changes observed in the distribution of urease are really the changes occurring in a single tissue, the parenchyma.

Upon this basis, the urease changes may be related to the aging of parenchyma cells. The urease content per unit volume of cell steadily decreases (fig. 7 A) as a cell ages. The decrease in urease units per gram of fresh weight is especially rapid during the period of cell elongation. After that, there is a slower decrease in the enzyme. This decrease is partially accounted for by the dilution of materials in the cell and by an increase in dry weight due to cellulose deposition, but there is also a definite inactivation or perhaps catabolism of urease taking place especially in the mature cells.

The metabolism of urease in the parenchyma cells may be briefly summarized (fig. 10 B) thus:

1. There is a rapid synthesis of urease in the actively dividing meristematic cells.
2. This synthesis continues during the period of cell elongation although at a decreasing rate, the amount of urease of a cell reaching a maximum at the end of the elongation period.

3. After this period, there is a decomposition of urease which continues down to a certain level.

A discussion of reasons for these changes will appear in the next paper of this series. The parenchyma cells of the root pass through the various growth stages and phases of urease change most rapidly, the stem cells less rapidly and the leaf cells least rapidly.

The cells possessing the densest cytoplasm contain the highest content of urease. This is true for the meristems of the root tip, stem apex, internodes, and flower promeristems. In the cotyledons the subepidermal parenchyma cells, the cells making up the bundle sheaths of the veins, and the young primary phloem cells, which appear to contain the densest cytoplasm, are also richest in urease. The density of cytoplasm is related to its protein content (the major portion of the protein being globulin). If the globulin urease undergoes changes similar to other globulins in synthesis and decomposition, it might be expected that an increase in protein content of the cytoplasm would be accompanied by an increase in urease activity. The data appear to confirm this.

The structures developing from the fertilized egg and laid down in the maturing seed have been treated separately from the structures which are differentiated after the seed germinates because the former possess a urease activity which is higher than that of similar organs developing in the seedling or mature plant. For example, at comparable stages of growth the plumule leaves (2 cm. long) have a urease activity per gram of fresh weight of over 15 times that of the ternate leaves of the same size; likewise, the young epicotyl (second internode) has a urease activity per gram of fresh weight of over 20 times that of an upper internode of similar weight. The cause of this difference in urease activity may be traced back to the development of the maturing seed, during which period there is a marked protein synthesis and storage including urease synthesis and storage. Upon germination, the meristematic cells of the radicle and plumule not only start with a high content of urease but are supplied by the cotyledons with a large amount of nitrogenous compounds from which to synthesize more urease during cell division and cell elongation. The later-developing structures are poorer in urease content, which is probably due to the fact that they are less richly supplied with nitrogenous compounds and must depend for their nitrogen on the mature and senescent cells and upon the supply of inorganic nitrogen obtained from the soil.

### Summary

1. The urease activity of *Canavalia ensiformis* has been determined using histological and quantitative methods. The data for total urease are summarized in figure 6 which is a graph showing the total urease content of the developing bean, and of the young plant through the 40-day-old stage. The distribution of urease in the various tissues of seedlings 10, 20, 28, and 40

days old is illustrated in a composite picture (figs. 1, 5). Stippling indicates the relative urease concentrations as revealed by the histological methods. The figures opposite each plant part express the urease activity in terms of urease units per gram of fresh weight as determined by the quantitative method. A description of the specific urease changes in the individual plant structures is given under their respective headings in the previous pages.

2. The changes observed in the urease content of the plant are due primarily to the changes in urease content of the parenchyma cells. Neither the cambium nor the cells derived from the cambium, that is, the phloem and xylem, contain any amounts of urease that can be detected with the indicator reagents.

3. The changes of urease activity in the parenchyma cells of any organ follow the same trends with aging of the cell. There is a rapid synthesis of urease in actively dividing cells. This synthesis continues during the elongation stage of the cell, the urease content of a cell reaching a maximum at the end of the elongation stage. After this stage, there is a decrease of the enzyme which continues until the urease content of the cell is down to a certain level.

4. The cells of the embryo developing from the fertilized egg and laid down in the maturing seed possess a urease activity which is higher than that of cells in similar organs developing in the seedling or mature plant.

The writer desires to express his gratitude to Professor F. G. GUSTAFSON for the many helpful suggestions and valuable criticisms throughout the course of this investigation and to Mr. HARMAN DUNHAM for assistance in taking the photomicrographs and in the preparation of the illustrations.

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NUTRITIONAL CHARACTERISTICS OF CERTAIN WOOD-  
DESTROYING FUNGI, *POLYPORUS BETULINUS*  
FR., *FOMES PINICOLA* (FR.) COOKE, AND  
*POLYSTICTUS VERSICOLOR* FR.

H. H. LAFUZE  
(WITH TWO FIGURES)

**Introduction**

Wood-destroying fungi vary greatly in their ability to attack different kinds of woods under natural conditions. A large number of them, such as *Polystictus versicolor* Fr., grow on a wide range of tree species. Many, however, are restricted to growth on a limited number of hosts, e.g., *Polyporus betulinus* Fr. attacks only birches, while *Fomes pinicola* (Fr.) Cooke is most commonly found on gymnosperms, and rarely on angiosperms. This specificity for certain substrates is obviously an inherent physiological characteristic of these fungi, conditioned by certain substances in woods. The experiments here reported are an attempt to determine some of the physiological differences between the wood-rotting forms, *Polyporus betulinus*, *Fomes pinicola*, and *Polystictus versicolor*. Emphasis is placed on the nutritional and growth characteristics shown by the organisms on various artificial media.

Investigations on durability of woods have suggested a number of factors which may be responsible for the selective growth of certain fungi on specific substrates. These factors, as commonly reviewed in the literature (1, 5, 6, 8, 18), include the presence of toxic water-soluble extractives such as phenolic compounds, alkaloids, and fatty acids, the waterproofing effect of resins, the toxicity of resins, pine oils, terpenes, quinones, and volatile materials, the specific gravity, moisture content, and air-water balance in wood, and the nutritional characteristics of woods, especially those of nitrogenous nature. Woods which have been subjected to high temperatures and long water treatments are invariably decayed to a greater extent than untreated woods (1, 10, 18). This loss of immunity to decay by the woods is undoubtedly caused by changes in water-soluble and volatile materials occurring in woods during water treatments and subsequent heating in an autoclave. Experiments have been included in this investigation to determine the influence of certain water-soluble substances, commonly present in woods, on the growth of the three fungi.

**Methods**

The strains of fungi used in these experiments were obtained from the Forest Products Laboratory of Madison, Wisconsin. They were received as



*Polystictus versicolor*, no. D. 1524, isolated from oak wood; *Fomes pinicola*, no. 610, isolated from a sporophore; and *Polyporus betulinus*, no. 652, isolated from sporophore tissue. Stock cultures of these were grown for two years preceding these experiments on media consisting of 1 per cent. Spramalt extract and 1.5 per cent. agar.

The basic components used in making experimental media and the exact formula of the control medium were as follows:

#### BASIC FORMULA, CONTROL MEDIUM

Carbohydrate, maltose .....	40 gm.
Nitrogen, Witte's peptone .....	4 gm.
Mineral salt, dipotassium acid phosphate .....	1 gm.
Substratum, agar-agar .....	15 gm.
Water, distilled water to make .....	1000 cc.

U.S.P. and C.P. chemicals were used in the preparation of the media, with a maximum error in weighing of 0.5 per cent. A complete series of media differing only in the quality of a single nutrient in the basic formula was prepared at one time. The different kinds of this variable nutrient were weighed into a series of small beakers. The remaining ingredients of the control medium were heated in a large beaker to melt the agar and dissolve the solutes. This was then apportioned quantitatively among the original small beakers of the series containing the variable component after which the contents were again heated and stirred thoroughly. The pH was adjusted between 5.5 and 6.0 by neutralizing with NaOH or HCl. The media were then tubed in dry test-tubes which had been thoroughly cleaned. The tubes were plugged with cotton and autoclaved at 15 pounds pressure and about 120° C. for 15 minutes. All tubes were slanted uniformly and allowed to cool.

The inoculum, consisting of a cube of substrate not exceeding eight cu. mm., was cut from six-day-old stock cultures growing on the malt extract-agar medium. Triplicate cultures for each fungus on each medium in a series were incubated in darkened cabinets at a temperature which varied between 24° and 28° C.

Data were recorded on the tenth day after inoculation. The extent of growth over the surface of the substrate was measured from the inoculum to the farthest edge of the mycelial mat and was expressed as millimeters of linear growth. This linear measurement of mycelial mats, which is commonly used as an index of growth, was considered inadequate by itself in expressing growth since it did not take into consideration the amount of mycelium produced per unit area of surface. This objection was overcome in part by allowing the arbitrary value of five to represent the compactness and thickness of the most luxuriant mycelial mats, such as those produced by *Poly-*

*stictus versicolor* on the control medium, and then estimating the relative thickness of the other mats in terms thereof. The ratings were made according to the following scale: 0 indicating no growth, 1 scanty, 2 thin, 3 good, 4 abundant, and 5 luxuriant growth. These values were referred to as rates of thickness of mycelium.

The woods used were *Tilia americana* L., *Betula nigra* L., *Betula papyrifera* Marsh., *Maclura pomifera* Schneid., *Quercus alba* L., *Pinus strobus* L., *Juniperus virginiana* L., *Juglans cinerea* L., and *Platanus occidentalis* L. Tree trunks about six inches in diameter were cut during June in eastern Iowa. Sawdust from each wood was ground in a pulp-mill to pass a 40-mesh sieve and stored in air-tight mason jars till used.

Aqueous extracts of the woods were made by adding about 50 gm. of the prepared sawdust to 750 cc. of distilled water, and autoclaving at 15 pounds pressure for 1.5 hours. The total volume of extract for each wood was evaporated to 450 cc. over a steam bath, thus producing an extract, 9 cc. of which represented about one gram of wood. A series of media was prepared by using the aqueous extracts of different woods instead of the water in the control medium. The formula of the control medium was further altered to include two per cent. agar. The approximate pH of each medium was determined.

Pieces of different woods measuring  $1/4 \times 3/4 \times 2$  inches were cut and trimmed until they were similar with respect to relative percentage of sapwood and heartwood, absence of knots and abnormalities, and uniformity in size and shape. After soaking in distilled water for 24 hours at 21° C., like species of wood were strung in groups of four on nichrome wire and suspended over 20 cc. of water in 250-cc. wide-mouthed Erlenmeyer flasks, which were stoppered with corks containing two pieces of glass tubing plugged with cotton. These flasks were then autoclaved at 15 pounds pressure for 15 minutes, and allowed to set in the laboratory for two weeks to test their sterility. They were finally divided into two lots and inoculated with *Poly-stictus versicolor*, and *Polyporus betulinus*, respectively. The cultures were incubated eight months in a dark room at a temperature of 22°–25° C. and a high relative humidity.

### Experimental results

#### CARBOHYDRATE NUTRITION

Various carbohydrates were substituted for maltose in the control medium. The fungi developed the greatest amount of mycelium in the presence of inulin, followed by dextrin, maltose, levulose, glucose, mannose, starch, and raffinose (table I). The least growth was observed in the presence of glucosides, and no growth occurred on lactose.

*Fomes pinicola* was the most variable of all the fungi in its growth on this

TABLE I

GROWTH OF THE FUNGI ON ARTIFICIAL MEDIA CONTAINING DIFFERENT CARBOHYDRATES (4.0%). LINEAR GROWTH REPRESENTS DISTANCE FROM INOCULUM TO EXTREMITY OF MYCELIAL MAT; RATE OF THICKNESS REPRESENTS THICKNESS OF MYCELIUM IN TERMS OF AN ARBITRARY VALUE (PAGE 627). PERCENTAGES FOR EACH FUNGUS ARE IN TERMS OF THE GROWTH OF THAT SPECIES ON THE CONTROL MEDIUM.\*

MEDIUM	<i>POLYPORUS BETULINUS</i>				<i>FOMES PINICOLA</i>				<i>POLYSTICTUS VERSICOLOR</i>			
	LINEAR GROWTH		THICKNESS		LINEAR GROWTH		THICKNESS		LINEAR GROWTH		THICKNESS	
	mm.	%	rate	%	mm.	%	rate	%	mm.	%	rate	%
None	6	38	1	50	22	76	1	20	28	65	1	20
Arabinose	18	112	2	100	22	76	2	40	39	91	4	80
Xylose	20	125	2	100	19	66	2	40	38	88	3	60
Rhamnose	10	62	1	50	15	52	1	20	33	77	1	20
Glucose	18	112	2	100	27	93	5	100	40	93	3	60
Levulose	18	112	2	100	27	93	5	100	43	100	4	80
Galactose	18	112	2	100	23	79	1	20	40	93	4	80
Mannose	18	112	2	100	24	83	5	100	42	98	4	80
Sucrose	21	131	2	100	28	97	2	40	41	95	4	80
Maltose*	16	100	2	100	29	100	5	100	43	100	5	100
Lactose	0	0	0	0	0	0	0	0	0	0	0	0
Raffinose	19	119	2	100	25	86	4	80	42	98	5	100
Dextrin	20	125	2	100	32	110	5	100	46	107	5	100
Inulin	30	187	2	100	33	114	3	60	51	118	5	100
Corn starch	16	100	2	100	31	107	5	100	40	93	5	100
Gum arabic	14	88	1	50	27	93	1	20	38	88	4	80
Gum tragacanth	21	131	2	100	26	90	5	100	35	81	2	40
Glycerin	16	100	2	100	16	55	3	60	31	72	4	80
Saponin	0	0	0	0	1	3	2	40	21	49	5	100
Salicin	9	56	2	100	15	52	1	20	16	37	4	80
Amygdalin	2	12	3	150	4	14	1	20	3	7	4	80

\* Control medium.

series of media. Pentoses and pentosans were especially poor nutrients, and the glucosides were definitely inhibitive to its growth. The most luxuriant growth was observed on glucose and its condensation products, dextrin, starch, and maltose, although good growth occurred on levulose, gum tragacanth, and mannose.

*Polystictus versicolor* grew relatively well in the presence of unusually high concentrations of glucosides, especially four per cent. saponin, which was quite toxic to the other wood-destroying fungi. Although the actual amount of mycelium produced by *Polyporus betulinus* on these media was very much less than that of the other fungi, it was more uniform in thickness. Contrasting with other fungi was the very extensive linear growth of the birch fungus in the presence of inulin and the complete inhibition of growth by saponin.

#### NITROGEN NUTRITION

Different nitrogen compounds were substituted for Witte's peptone in the control medium. A general survey of the data shows that the best growth of the fungi was obtained on media containing, in order of diminishing amounts, proteins, peptones, amino acids, and inorganic nitrogenous salts (table II). The best inorganic source of nitrogen was ammonium nitrate. There was only slight indication that ammonia may be better utilized than nitrate. Sodium nitrite and urea were apparently non-nutritive to all three fungi. The monamino-dicarboxylic acids, aspartic and glutamic, supported good fungal growth, while glycine, a monocarboxylic acid, was a poor nutrient. Growth was almost completely inhibited by the two amino acids, tyrosin and cystine, which contain phenyl and disulphide radicals, respectively.

Characteristic of the fungi was the luxuriant growth on gliadin, a protein which contains relatively large amounts of ammonia, tryptophane, and glutamic acid. Less abundant growth occurred on glutenin and casein, both of which are low in glutamic acid, ammonia, and tryptophane content but contain relatively more tyrosin than the other proteins. The hydrolysate of casein, deficient in tryptophane, consistently produced less mycelium than casein itself. It was found that nitrogen sources of high molecular weight, those containing tryptophane, ammonia, and glutamic acid and those low in tyrosin content favor optimum growth of the fungi.

*Fomes pinicola* was most tolerant to the variety of nitrogen sources supplied in this series of media. Exceptionally good growth occurred in the presence of the ammonium and nitrate salts. The organism developed better on cystine and glycine than either of the other fungi. In contrast, *Polyporus betulinus* grew poorly on this series of media. Striking variations in its growth as compared with the other fungi were noticed in the greatly increased thickness, but much reduced linear growth of mycelium, in the retardation of



growth on tryptophane-deficient casein hydrolysate and gelatin, as well as on glutenin, egg albumin, and particularly cystine, and in the inhibition of growth by both sodium nitrate and ammonium chloride as compared with media lacking any nitrogen source.

### Mineral nutrition

The formula of the control medium was varied by substituting different salts for the dipotassium acid phosphate at the same concentration, one gram per liter of medium. Potassium and phosphate ions were essential for good growth of the fungi (table III). The presence of potassium was correlated with a slight increase in the spread of mycelium, while that of phosphate was correlated with a conspicuous increase in thickness of mycelial mats. Of secondary importance to potassium and phosphate was calcium, which generally caused an increase in growth, particularly of *Fomes pinicola*. The presence of sodium ions appeared to retard growth especially if the spread of mycelium be taken as a criterion. This was noticeable also in the tripotassium acid phosphate medium which had been neutralized with sodium hydroxide. The medium to which no mineral salts were added supported fungal growth equal to that observed on other media containing salts, suggesting that nutritive salts might have been introduced into the first medium as an impurity of some other ingredient.

Growth of *P. betulinus* was especially scanty when compared with that of the other species. Both the birch and conifer fungi were more variable in growth responses to different mineral salts than *P. versicolor*, further emphasizing the specificity of *P. betulinus* and *F. pinicola* for certain nutrients.

### EFFECT OF PH ON GROWTH

The acidity of a short series of media made from the formula of the control medium was varied from pH 4 to 8 by adding hydrochloric acid or sodium hydroxide. The best growth of the three fungi was on an acid medium of pH 5.6, determined with the aid of a Lamotte Roulette Comparator. The three species did not differ noticeably in their pH requirements for optimum growth. Acid media tended to cause greater spread of mycelium and thinner mycelial mats than more alkaline media.

A series of media was prepared in which acidity changes of the substrate could be observed during the growth of the fungi. Different indicators were added to respective 6-cc. portions of the control medium in the following concentrations:

6	drops	of	0.01	per	cent.	methyl	orange
4	"	"	0.01	per	cent.	Congo	red
6	"	"	0.02	per	cent.	methyl	red
4	"	"	0.01	per	cent.	neutral	red

TABLE III

GROWTH OF THE FUNGI ON ARTIFICIAL MEDIA CONTAINING DIFFERENT MINERAL SALTS (0.1%). LINEAL GROWTH REPRESENTS DISTANCE FROM INOCULUM TO EXTREMITY OF MYCELIAL MAT; RATE OF THICKNESS REPRESENTS THICKNESS OF MYCELIUM IN TERMS OF AN ARBITRARY VALUE (PAGE 627). PERCENTAGES FOR EACH FUNGUS ARE IN TERMS OF THE GROWTH OF THAT SPECIES ON THE CONTROL MEDIUM\*

MEDIUM	<i>POLYPORUS BETULINUS</i>				<i>FOMES PINICOLA</i>				<i>POLYSTICTUS VERSICOLOR</i>			
	LINEAR GROWTH		THICKNESS		LINEAR GROWTH		THICKNESS		LINEAR GROWTH		THICKNESS	
MINERAL SALT	mm.	%	rate	%	mm.	%	rate	%	mm.	%	rate	%
None .....	15	63	2	66	24	80	2	40	42	93	2	40
KH <sub>2</sub> PO <sub>4</sub> .....	21	88	3	100	30	100	5	100	50	111	5	100
K <sub>2</sub> HPO <sub>4</sub> * .....	24	100	3	100	30	100	5	100	45	100	5	100
K <sub>2</sub> PO <sub>4</sub> .....	3	13	5	166	19	63	5	100	35	78	5	100
Na <sub>2</sub> HPO <sub>4</sub> .....	2	8	5	166	7	23	3	60	31	68	5	100
CaHPO <sub>4</sub> .....	15	63	3	100	24	80	4	80	40	89	5	100
KCl .....	13	54	3	100	26	87	2	40	43	96	2	40
NaCl .....	11	46	2	66	20	67	2	40	42	93	2	40
K <sub>2</sub> SO <sub>4</sub> .....	12	50	2	66	28	93	2	40	42	93	2	40
Na <sub>2</sub> SO <sub>4</sub> .....	12	50	2	66	23	77	2	40	41	91	2	40
CaSO <sub>4</sub> .....	10	42	2	66	25	83	4	80	47	103	2	40
CaCl <sub>2</sub> .....	11	46	2	66	21	70	3	60	43	95	2	40

\* Control medium.

5	"	"	0.04 per cent. bromphenol blue
4	"	"	0.04 per cent. bromcresol purple
4	"	"	0.04 per cent. bromthymol blue

The original pH of the medium was about 5.6. The changes in color occurring during growth of the fungi were observed under the periphery and the older portions of the mycelial mats.

There was a general tendency toward increased acidity in cultures of all three fungi. This was particularly noticed by a change in color of the indicators slightly in advance, but mostly directly beneath, the very young and actively growing mycelium. The colors due to indicators disappeared under the older mycelium. This was attributed to the alteration or destruction of the indicator by the organisms, since acids and alkalies added to the substrates caused no reappearance of the indicator color. The substrates of *Fomes pinicola* became the most acid, estimated to be about pH 3. Those of *Polyporus betulinus* were almost as acid, while the *Polystictus versicolor* substrates remained strikingly more alkaline, or about pH 4.5.

#### OXIDATION-REDUCTION PHENOMENA

Oxidation and reduction indicators were added to respective portions of media prepared from the formula of the control medium. Oxidation was observed in the media growing *Polystictus versicolor* and containing guaiacol, resorcinol, and tannin, while a doubtful reaction was obtained in media containing tyrosin, and quinol (table IV). Of the other two fungi, only *F. pinicola* showed a positive reaction with any of the oxidation indicators, producing but slight coloration in the tyrosin medium.

TABLE IV

OXIDATION AND REDUCTION REACTIONS OF THE FUNGI GROWN ON THE CONTROL MEDIUM IN THE PRESENCE OF INDICATORS. NO GROWTH IS INDICATED BY 0, NO REACTION BY -, WEAK POSITIVE REACTION BY +, MODERATE POSITIVE REACTION BY ++, AND STRONG POSITIVE REACTION BY +++.

MEDIUM INDICATOR	<i>POLYPORUS BETULINUS</i>	<i>FOMES PINICOLA</i>	<i>POLYSTICTUS VERSICOLOR</i>
0.1 per cent. guaiacol .....	0	-	+
0.02 " " <i>α</i> -naphthol .....	0	0	0
0.1 " " tyrosin .....	-	+	++
0.02 " " pyrogallol .....	0	0	0
0.1 " " resorcinol .....	-	-	+++
0.1 " " tannin .....	-	-	+++
0.02 " " methylene blue	+++	-	-
0.002 " " methylene blue	+++	++	+



TABLE V

GROWTH OF THE FUNGI ON ARTIFICIAL MEDIA CONTAINING DIFFERENT PHENOLIC COMPOUNDS (0.02%) IN ADDITION TO NUTRIENTS OF THE CONTROL MEDIUM. LINEAR GROWTH REPRESENTS DISTANCE FROM INOCULUM TO EXTREMITY OF MYCELIAL MAT; RATE OF THICKNESS REPRESENTS THICKNESS OF MYCELIUM IN TERMS OF AN ARBITRARY VALUE (PAGE 627). PERCENTAGES FOR EACH FUNGUS ARE IN TERMS OF THE GROWTH OF THAT SPECIES ON THE CONTROL MEDIUM\*

MEDIUM PHENOLIC COMPOUND	<i>POLYPORUS BETULINUS</i>			<i>FOMES PINICOLA</i>			<i>POLYSTICTUS VERSICOLOR</i>		
	LINEAR GROWTH		THICKNESS	LINEAR GROWTH		THICKNESS	LINEAR GROWTH		THICKNESS
	mm.	%	rate	mm.	%	rate	mm.	%	rate
None*	17	100	3	30	100	4	43	100	5
Guaiacol	2	12	4	10	33	5	42	98	5
Resorcinol	16	94	3	27	90	3	34	80	5
Quinol	15	88	3	31	103	4	12	28	5
Pyrogallol	0	0	0	0	0	0	0	0	0
$\alpha$ -Naphthol	0	0	0	0	0	0	0	0	0
Tannin	17	100	3	20	66	5	47	108	5

\* Control medium.

Reduction of methylene blue to the leuco-compound occurred in *F. pinicola* and *P. betulinus* cultures. *F. pinicola* was the most active in reducing this indicator. The whitened substrate soon regained a deep blue color which later changed to green, due to re-oxidation of the leuco-compound. The medium in *P. betulinus* cultures remained white for three weeks without indication of re-oxidation of the indicators. *P. versicolor* decolorized methylene blue only after cultures had been kept two weeks. The reaction, however, was limited to immediately below the mycelial mat and was almost immediately followed by an oxidation reaction, producing a green color.

Oxidase studies were continued by treating portions of the mycelium and the substrate on which the fungi had grown with a solution of guaiacum. The substrate of *Polystictus versicolor* cultures gave a strong blue color in the presence of the oxidase indicator, while the mycelium showed a weaker color reaction but nevertheless positive. Neither the mycelium nor the substrates of *F. pinicola* and *P. betulinus* produced a positive test for oxidase.

#### GROWTH IN THE PRESENCE OF PHENOLIC COMPOUNDS

Different phenolic compounds were added to respective portions of a medium made from the formula of the control medium. The pH was not adjusted. No growth occurred in the presence of 0.02 per cent. alpha-naphthol and pyrogallol (table V). The presence of the two dihydric phenols,

TABLE VI

GROWTH OF THE FUNGI ON ARTIFICIAL MEDIA CONTAINING DIFFERENT AMOUNTS OF TANNIN IN ADDITION TO NUTRIENTS OF THE CONTROL MEDIUM. LINEAR GROWTH REPRESENTS DISTANCE FROM INOCULUM TO EXTREMITY OF MYCELIAL MAT; RATE OF THICKNESS REPRESENTS THICKNESS OF MYCELIUM IN TERMS OF AN ARBITRARY VALUE (PAGE 627). PERCENTAGES FOR EACH FUNGUS ARE IN TERMS OF THE GROWTH OF THAT SPECIES ON THE CONTROL MEDIUM\*

TANNIN		<i>POLYPORUS BETULINUS</i>				<i>FOMES PINICOLA</i>				<i>POLYSTICTUS VERSICOLOR</i>			
MEDIUM		LINEAR GROWTH		THICKNESS		LINEAR GROWTH		THICKNESS		LINEAR GROWTH		THICKNESS	
%		mm.	%	rate	%	mm.	%	rate	%	mm.	%	rate	%
0.0*		20	100	2	100	25	100	3	100	42	100	5	100
0.01		23	115	2	100	25	100	3	100	44	105	5	100
0.05		22	110	2	100	22	88	3	100	38	90	4	80
0.10		18	90	2	100	20	80	3	100	18	43	2	40
0.15		15	75	2	100	19	76	3	100	0	0	0	0
0.20		15	75	2	100	20	80	3	100	0	0	0	0
0.30		12	60	2	100	12	48	4	133	0	0	0	0
0.40		7	35	3	150	12	48	4	133	0	0	0	0

\* Control medium.

resorcinol and quinol, retarded the growth of *P. versicolor*, while *F. pinicola* and *P. betulinus* were retarded appreciably only in the presence of the methylated catechol, guaiacol.

Tannin at a concentration of 0.15 per cent. completely inhibited the growth of *P. versicolor*, while the other fungi continued to grow at a concentration of 0.4 per cent. (table VI). The substrate of *P. versicolor* turned to a deep brown color in the presence of tannin, a phenomenon which suggests that oxidation products of tannic acid may be a factor inhibiting growth. With the increase in concentration of tannin, the ratio of surface area over thickness of mycelium of *F. pinicola* and *P. betulinus* increased.

#### GROWTH ON AQUEOUS EXTRACTS OF WOODS

Cultures were made on a series of media containing aqueous extracts of different woods. The control medium was the same as that used in preceding experiments.

Black and white birch extracts did not inhibit the growth of any of the fungi, in fact the development of mycelium was increased (table VII, fig. 1). On the other hand, white pine and osage orange extracts, both containing a

TABLE VII

GROWTH OF THE FUNGI ON ARTIFICIAL MEDIA CONTAINING AQUEOUS EXTRACTS OF DIFFERENT WOODS IN ADDITION TO THE NUTRIENTS OF THE CONTROL MEDIUM. LINEAR GROWTH REPRESENTS DISTANCE FROM INOCULUM TO EXTREMITY OF MYCELIAL MAT; RATE OF THICKNESS REPRESENTS THICKNESS OF MYCELIUM IN TERMS OF AN ARBITRARY VALUE (PAGE 627). PERCENTAGES FOR EACH FUNGUS ARE IN TERMS OF THE GROWTH OF THAT SPECIES ON THE CONTROL MEDIUM\*

MEDIUM WOOD EXTRACT	<i>POLYPORUS BETULINUS</i>				<i>FOMES PINICOLA</i>				<i>POLYSTICTUS VERSICOLOR</i>			
	LINEAR GROWTH		THICK- NESS		LINEAR GROWTH		THICK- NESS		LINEAR GROWTH		THICK- NESS	
	mm.	%	rate	%	mm.	%	rate	%	mm.	%	rate	%
None* .....	20	100	3.0	100	25	100	2.7	100	41	100	4.0	100
White pine .....	0	0	0	0	3	12	5.0	185	30	73	4.3	107
Basswood .....	15	75	3.3	110	20	80	4.0	144	42	102	4.7	117
White oak .....	12	60	3.0	100	8	32	4.3	159	36	88	5.0	125
White birch .....	20	100	3.7	123	23	92	4.0	144	45	109	4.3	107
Butternut .....	19	95	3.7	123	23	92	4.0	144	26	63	5.0	125
Osage orange ...	0	0	0	0	0	0	0	0	1	3	2.0	50
Sycamore .....	20	100	3.7	123	16	64	4.0	144	43	106	4.3	117
Black birch .....	25	125	3.7	123	27	108	4.0	144	43	106	4.7	117
Red cedar .....	18	90	3.7	123	25	100	4.0	144	38	92	5.0	125

\* Control medium.

colloidal suspension unlike that in the other extracts, were strikingly inhibitive to the growth of the three fungi, especially to *P. betulinus*, and also but to a less extent, to *F. pinicola*.

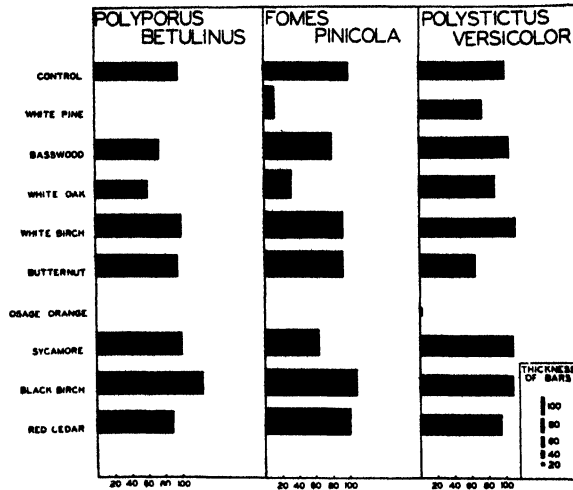


FIG. 1. Growth of the fungi on artificial media containing aqueous extracts of different woods in addition to the nutrients of the control medium. Length of bars represents linear growth, thickness of bars represents thickness of mycelium. Percentages for each fungus are in terms of the growth of that species on the control medium.

The reddish brown extracts of white oak, butternut, basswood, and red cedar differed in their influence on the development of the mycelium of each fungus. *P. versicolor* was retarded by extracts of white oak and butternut, yet it grew better than, or equally as well as, the other fungi on white oak, basswood, birch, and sycamore. Extracts of basswood, red cedar, and white oak definitely retarded growth of *P. betulinus*, while black birch extract supported better growth of the birch fungus than any other extract. *F. pinicola* was inhibited by basswood, white oak, butternut, and sycamore extracts. The best growth of this fungus occurred on black birch extract, although the organism grew better on red cedar than either of the other fungi, and better on white pine than *P. betulinus*.

#### GROWTH OF THE FUNGI ON WOODS

*Polystictus versicolor* and *Polyporus betulinus* were cultured on a variety of woods under laboratory conditions. Both fungi grew luxuriantly on sycamore, black and white birch, and basswood, but very poorly on white oak, butternut, white pine, osage orange, and red cedar (fig. 2). The first group of woods named were light colored while the latter group with the exception of white pine possessed dark-colored heartwood due to the presence

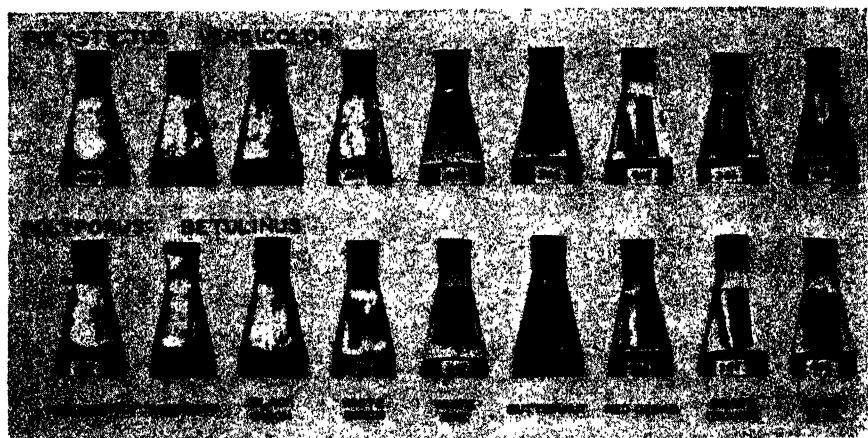


FIG. 2. Eight months old cultures of *Polystictus versicolor* and *Polyporus betulinus* on different woods.

of colored materials, and light-colored sapwood. The mycelia of the fungi were seldom found on the dark colored heartwoods, and then only very scantily. The light colored sapwoods invariably supported fair growth of mycelium, thus indicating that growth was retarded by the presence of substances found in the dark colored heartwoods of certain trees. The fungi did not differ markedly from each other in extent of growth, although the birch fungus produced less mycelium than *P. versicolor*.

### Discussion

The chief problem in these experiments was the determination of outstanding nutritional differences and growth contrasts of *Polystictus versicolor*, *Fomes pinicola*, and *Polyporus betulinus* on artificial media, in order to gain a clue to the nature of the specificity of the latter two organisms for certain substrates. It was found that variations in the kind of carbohydrate, nitrogen, and mineral constituents of artificial media caused differences in the growth of the fungi, varying from mycelial mats spreading over the entire surface of the substrate to none at all, and from very thin to thick and luxuriant growths. The addition of phenolic compounds and aqueous extracts of woods to artificial nutrient media inhibited or increased in varying degrees the growth of each fungus. The activity of oxidases and reductases of the fungi in the presence of indicators was also different for each species. Growth of *P. versicolor* and *P. betulinus* was observed on a variety of woods but no specific differences between the fungi were noticed.

Difficulty in interpreting data and comparing them with those of other investigators was experienced because of certain variable factors which were believed to influence growth of fungi. Woods collected in different seasons,

or from different regions, and those dried or prepared for cultures under different conditions may cause significant variations in development of the fungi growing on them or on their extracts. Chemicals obtained from different sources and used in preparing nutrient media may influence differently the development of fungi. SCHOPFER (14) discovered that impurities present in some maltoses and absent in others were essential for reproduction of certain phycomycetes. Differences in growth characteristics observed by SCHMITZ (13) with strains of *Fomes pinicola* isolated from different wood hosts suggested the necessity of knowing the wood host from which the fungus was isolated and also the nature of the stock culture medium on which the fungus was grown previous to the experiment. Significant changes occurring in woods during the preparation of laboratory cultures and probably involving among other things leaching and chemical or physical modifications of wood substances not only may vary with different procedures used by investigators and thereby modify growth data, but also may cause growth phenomena in laboratory cultures which are different from those occurring in nature. Characteristics common for a fungus growing on artificial substrates, such as the agar gel, were regarded as not necessarily being in correlation with those of the same fungus growing on woody substrates which are more complex physically and chemically. Also considered very significant and probably affecting the development of the fungi were the differences in the environments of nature and of the sterile, humid, enclosed, and temperature-controlled laboratory culture chambers.

*Polystictus versicolor* was very profuse in its growth, and showed evidences of possessing much vitality and hardiness on a wide variety of nutrient media, and in the presence of many toxic substances, including glucosides, phenolic compounds, and extracts of woods. Neither *F. pinicola* nor *P. betulinus* showed such great adaptability to this variety of substrates. The coefficient of variability of growth over the surface of the control medium in these experiments was 3.7 per cent. for *P. versicolor*, while those for *F. pinicola* and *P. betulinus* were three to four times greater, 10.7 and 13.4 per cent., respectively (table VIII). The average linear growth of *F. pinicola* and *P. betulinus* was only 64 and 45 per cent., respectively, of the growth of *P. versicolor* on similar lots of control media. It becomes apparent, then, that *P. versicolor* can adapt itself to many different substrates while the other two fungi are much less adaptive and may be inhibited in growth by certain substances or combinations of substances present in the substrates.

LUTZ (10) reported that neither *Ungulina betulina* (= *Polyporus betulinus*) nor *Polyporus pinicola* (= *Fomes pinicola*) would grow on oak wood unless the wood were first extracted with water to remove substances inhibitive to the growth of the fungi. SNELL (17) and LIESE (9) found that *Polyporus betulinus* grew equally well and caused similar amounts of decay in

TABLE VIII

COMPARISON OF THE GROWTH OF THE FUNGI CULTURED AT DIFFERENT TIMES ON THE CONTROL MEDIUM. LINEAR GROWTH REPRESENTS DISTANCE FROM INOCULUM TO EXTREMITY OF MYCELIAL MAT; RATE OF THICKNESS REPRESENTS THICKNESS OF MYCELIUM IN TERMS OF AN ARBITRARY VALUE (PAGE 627)

CONTROL MEDIUM	<i>POLYPORUS BETULINUS</i>		<i>FOMES PINICOLA</i>		<i>POLYSTICTUS VERSICOLOR</i>	
	LINEAR GROWTH	THICKNESS	LINEAR GROWTH	THICKNESS	LINEAR GROWTH	THICKNESS
no.	mm.	rate	mm.	rate	mm.	rate
208 .....	20	2	29	5	43	5
	15	2	28	5	44	5
	16	2	30	5	42	5
318 .....	17	3	22	4	40	4
	20	3	23	4	42	4
	20	3	24	5	40	4
403 .....	25	3	30	5	45	5
	24	3	30	5	45	5
	22	3	30	5	....	...
620 .....	20	3	24	2.7	42	4
	18	3	25	2.7	40	4
500 .....	17	3	30	4	44	5
	17	3	30	4	43	5
	18	3	30	4	42	5
800 .....	20	2	25	3	42	5
	20	2	25	3	42	5
Total .....	309		435		636	
Mean .....	19.3		27.2		42.4	
Percentage mean in terms of mean of <i>P. versicolor</i> ..	45		64		100	
Standard deviation .....	2.6		2.9		1.6	
Coefficient of variability .....	13.4		10.7		3.7	

laboratory cultures on oak, basswood, certain conifers, and birch woods. Similar results were obtained by the author with the same fungus species on sycamore, basswood, and birch which were in contrast with poor growth of the fungus on white oak, butternut, osage orange, red cedar, and white pine. That the substances present in woods and inhibitive to growth of certain fungi are water soluble and can be removed by water treatments was further suggested in the work of SOWDER (18), ANDERSON (1), and HAWLEY, FLECK, and RICHARDS (6) and was evident in these experiments from the exceptionally good growth of *P. betulinus* on aqueous extracts of birch and the definite

inhibition of growth by extracts of white oak, osage orange, red cedar, white pine, and basswood.

Water soluble substances in woods which are best known for their toxic effects on wood-destroying fungi are the phenolic compounds. Either *P. betulinus* or *F. pinicola* was inhibited to some extent by 0.02 per cent.  $\alpha$ -naphthol, pyrogallol, resorcinol, and guaiacol in artificial media. Growth of these fungi was strongly retarded by 0.3 and 0.4 per cent. tannin. It follows that higher concentrations of tannin such as those present in some woods would also inhibit the growth of these wood-destroying fungi and thus be a factor responsible in part for the specificity of fungi for certain substrates (2, 10). *P. versicolor*, however, which is a common saprobe on many woods containing tannins, and thus apparently tolerant of phenolic compounds, was completely inhibited on artificial media in the presence of 0.15 per cent. or more of commercial tannin. An examination of the cultures of this fungus grown on substrates containing phenolic compounds or extracts of woods known to contain soluble phenolic substances showed an inhibition of growth of the fungus and a coloration beneath the mycelium of the organism, caused by the presence of oxidation products of the phenolic compounds. Since tannin and similar compounds present in substrates are inhibitive to the growth of the widely occurring organism, *P. versicolor*, as well as to that of the specific fungi, *P. betulinus* and *F. pinicola*, it follows that the presence of phenolic compounds is not the only factor which may determine the specificity of these fungi for certain woods in nature.

Glucosides, such as saponin and salicin, were not only in general inhibitive to the growth of fungi but they also varied in their toxic effects on each species. Growth of *P. betulinus* was completely inhibited by saponin, in contrast with the behavior of *P. versicolor*. Salicin favored relatively good growth of *P. betulinus*, and *F. pinicola* was retarded in growth by all of the glucosides. These observations not only suggest the inclusion of glucosides among the list of causal factors for specificity of fungi but also their very great importance as factors of growth and development of wood-destroying fungi on woods which may vary greatly in their glucosidic contents.

Differences in growth induced by various nutrients in artificial media were noticeable in cultures of all three fungi. *F. pinicola* exhibited relatively poor growth on carbohydrate media, especially in the presence of pentoses, galactose, and certain other carbohydrates which supported good growth of the other two fungi. The least variation in growth of this fungus on any series of nutrients was on media varying in kinds of nitrogen, where it grew equally well on inorganic nitrogen salts, amino acids, and proteins. In a contrasting manner, *P. betulinus* showed very good growth on the variety of carbohydrate media, but grew in varying amounts on the different nitrogen and mineral salt nutrients. Production of mycelium by



this fungus was especially less in the absence of potassium, phosphates, and nitrogenous compounds containing tryptophane, and in the presence of inorganic nitrogen salts and glycine, cystine, and tyrosin which were toxic in their effects on growth. These specificities toward nutrients which either inhibit or appear essential for good growth of the fungi in laboratory cultures suggest that a similar specificity may also occur on the widely different woody substrates in nature.

Studies of chemical changes in woods due to decay by fungi have been confined largely to soluble extractives, lignin, cellulose, and pentosans. The "proximate analysis" of apple wood rotted by *Polystictus versicolor* indicated that lignin was not attacked (16). However, BAYLISS (3), using microchemical methods, and CAMPBELL (4), using more precise analytical methods, distinguished *P. versicolor* from brown-rot fungi by its delignifying action on woods. CAMPBELL reported pentoses were removed first, then lignin was attacked, and finally, in the presence of increased acidity, cellulose. The rots produced by *Fomes pinicola* (11) and *Polyporus betulinus* (7) were described as brown rots in which relatively more cellulose than lignin was removed.

The majority of brown-rot fungi listed in a recent pathology text (8) were reported as occurring on gymnosperms, birches, and alders, and more than two-thirds of the white-rot fungi were described as commonly attacking angiosperms. Analyses, although incomplete for thorough comparisons of nutritive characteristics, show that gymnosperm woods differ markedly in chemical composition from angiosperm woods. To exemplify, an analysis of the data of RITTER and FLECK (12) and of SCHORGER (15) showed that the angiosperm woods analyzed by them contained among other and even greater differences 100 per cent. more pentosan than gymnosperm woods. Growth of the conifer fungus, *F. pinicola*, on pentoses and pentosans in artificial media was correspondingly much less than that of the two angiosperm-inhabiting fungi on similar media. These observations suggest that both quantitative and qualitative differences in nutrients existing among species of woods may be of considerable physiological importance to the successful growth and reproduction of fungi on woody substrates.

Since only three species of fungi were studied in these experiments, one cannot generalize too freely from the data. It is interesting to note, however, the correlation between the oxidation-reduction reactions of the fungi and their growth characteristics. *P. versicolor*, generally recognized as readily attacking phenolic compounds such as tannin and lignin, and being very wide spread in distribution on angiosperm and gymnosperm woods, possessed strong oxidases. *P. betulinus*, however, not extensively attacking tannin or lignin, and exhibiting a specificity only for birch woods, possessed strong reductases and no oxidases; while *F. pinicola*, which is specific for

gymnosperm woods but occasionally is found on certain angiosperms, possessed strong reductases and some oxidases. These data are indicative of the importance of respiratory enzymes in the phenomena of specificity of fungi for certain substrates which may lack essential respirable nutrients or contain toxic substances inhibitive to respiration and growth.

The differences in nutrition and growth noted herein are considered quite suggestive as to the causes of specificity among wood-rotting fungi. Needless to say, this cause is most likely a factor-complex involving physical, chemical, and biological characteristics of the substrates and the fungi. It was shown that the nature of carbon and nitrogen sources influenced the growth of wood-rotting fungi. The absence of certain nutrients or growth factors in woods may retard initial development and subsequent assimilative growth either entirely or to the extent that the fungus may never form a fructification by which the presence of the organism in woods is commonly recognized. Antagonistic substances present in woods may similarly affect nutrition, respiration, or reproduction. Principal among these antagonistic substances inhibitive to growth and studied in this investigation were glucosides and phenolic compounds.

### Summary

1. The nutrition of *Polystictus versicolor* Fr., *Fomes pinicola* (Fr.) Cooke, and *Polyporus betulinus* Fr. was studied by observing the growth of these fungi on artificial media varying qualitatively in nutrients, and on nutrient media containing phenolic compounds, aqueous extracts of woods, and oxidation-reduction and pH indicators, and on woods.

2. Certain nutritional characteristics were common to all three species:

(a) Polysaccharides supported the best growth of mycelium. Pentoses generally were not utilized as efficiently as hexoses. No growth occurred on lactose.

(b) Amino and ammonia nitrogen was better utilized than amide, nitrate, and nitrite nitrogen. The best growth was observed on proteins containing relatively large amounts of glutamic acid and tryptophane. Amino acids containing phenyl and disulphide radicals retarded growth.

(c) Potassium and phosphate salts were essential for optimum growth. Phosphate was associated with increased thickness of mycelial mats, and potassium with increased linear growth of mycelium.

(d) Glucosides and phenolic compounds retarded growth.

(e) Aqueous extracts of white pine and osage orange were especially toxic, while those of black and white birch were non-toxic and even appeared to accelerate growth. Other wood extracts influenced the growth of the fungi in varying ways.

3. *P. versicolor* invariably grew luxuriant mycelial mats on artificial media, and showed only a little selection for kinds of nutrients. It utilized

both salicin and saponin as a source of carbohydrate, and was most tolerant to the greatest number of aqueous extracts of woods. Growth of *P. versicolor*, like that of *P. betulinus*, was relatively poor on inorganic and amino nitrogen media. Unlike the other two fungi, its growth was retarded by the presence of quinol and tannin.

4. *F. pinicola*, in comparison with *P. versicolor*, formed less abundant mycelial mats with a much greater variability on different media. It was especially sensitive to differences in the carbohydrates, growing poorest on media containing pentoses, pentosans, galactose, and sucrose, while carbohydrates containing glucose produced in general the best growth. *F. pinicola* grew relatively well on inorganic nitrogen salts, amino acids, and proteins alike. It was inhibited by both saponin and salicin, and, like *P. betulinus*, by guaiacol. Growth on aqueous extracts of woods was most variable, being best on red cedar and birch extracts.

5. *P. betulinus* produced relatively thin and often scanty mycelial mats and of all three species showed the greatest variability in growth. It was most sensitive to differences in nitrogen sources, only gliadin, Witte's peptone, casein, and glutenin producing a good growth of mycelium. Growth on carbohydrates was like that of *P. versicolor*, very uniform. Poor growth occurred in the absence of the less acidic potassium phosphates, and in the presence of guaiacol and saponin. Aqueous extracts of white pine and osage orange inhibited growth, those of white oak, red cedar, and basswood greatly retarded growth, and birch extracts accelerated growth.

6. *P. betulinus* grew equally well on heartwoods of sycamore, basswood, and birch, on sapwoods of white oak, butternut, and osage orange, and on white portions of red cedar wood. *P. versicolor* differed only in producing more luxuriant mycelial masses than the birch fungus.

7. The presence of a strong oxidase system was recognized in *P. versicolor* by its oxidation of tannin, resorcinol, quinol, tyrosin, and guaiacol. Oxidases were very weak in *F. pinicola* and not detectable in *P. betulinus*.

8. Reductases were especially abundant in *F. pinicola* and *P. betulinus*, but apparently weak in *P. versicolor*.

9. Possible causes of specificity of these fungi on wood in nature are the absence of essential nutrients, and the presence of toxic substances in woods which may influence initial development, respiration, or fructification of the fungi.

10. It was possible to distinguish physiologically between these (a) gymnosperm-inhabiting and angiosperm-inhabiting fungi, (b) white-rot producing and brown-rot producing fungi, and (c) fungi specific and those not specific for certain woods in nature.

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# METHODS OF SAMPLING VISIBLE RADIATION<sup>1</sup>

RAYMOND H. WALLACE

(WITH TEN FIGURES)

## Introduction

There is perhaps no factor in the measurement of light which is more essential to the correct evaluation of light records and their correlation with various biological phenomena than the method of sampling of this radiation. Yet a survey of the literature on the subject discloses that no comprehensive tests have been made on the various possible physical arrangements of the sensitive surfaces of the receiving units and the degree of similarity which these various combinations will exhibit when checked by simultaneous readings of the same light station. We are all aware that the method so commonly used of exposing a single-plane receiving surface in one direction gives erratic results. Thus any one that has used any of the numerous exposure meters or illuminometers, having a flat surface as the receiving unit, knows from experience that a reading may vary from a few to perhaps almost 100 per cent. if the receiving unit is tilted a few degrees. This indicates a fundamental flaw in the sampling, for the original value will be found when the first position is again obtained. It would seem, therefore, that we must, if we are to use light records with any degree of success, have methods of sampling that make impossible these great fluctuations in value.

AURENS (1) working on the light habitats for various regions in Sweden, has taken great care to insure that the sampling method used checks perfectly with the method in use by the weather bureau for their determinations of total radiation. In his test the photocell is mounted in a chamber having a single round window in the top to admit the light to be measured. This window is fitted with a piece of opal glass which is ground on both sides to insure that the optics of the arrangement obey Lambert's cosine law. This unit is, of course, mounted horizontally in accordance with weather bureau practice. His method can be said, therefore, to duplicate the weather bureau practice with an instrument intended to measure visible radiation for use in work on silviculture and ecology. Apparently no tests were made to determine the suitability or dependability of this method for work of this type. It is obvious that a method which may be perfectly adapted to determinations of solar constants and other highly specialized factors of interest to those in meteorology may be quite unsuited for ecological or physiological studies.

<sup>1</sup> This paper was presented before the symposium on the measurement of light, held under the auspices of the New England Section of the American Society of Plant Physiologists at Connecticut State College, May 8 and 9, 1936.

The possible lack of suitability of radiation records of this type as applied to silvical research has been recognized by GAST (2) in his studies on the effect of total radiation on the growth of pine. He states as follows: "Further, in order to give a time record it (the receiving unit) must integrate the rays coming from all directions at normal incidence." In attempts to attain this condition GAST has designed and constructed four types of radiometers made up of different combinations of spherical junction thermocouples. His best type, type D, integrates the radiation with an accuracy of  $\pm 9$  per cent. for angles of incidence from  $5^\circ$  to  $80^\circ$ . For the entire range from  $0^\circ$  to  $90^\circ$  the values are as follows: For  $90^\circ$  the relative sensitivity is 74; for  $60^\circ$ , 78; for  $30^\circ$ , 86; for  $10^\circ$ , 94; for  $5^\circ$ , 95; and for  $0^\circ$ , 100.

It is in response to this need of more definite data than are now available on methods of sampling that the series of tests reported in this paper were made. I shall limit myself in the present paper to sampling of radiation from a light station which is unobstructed by surrounding objects and therefore has the horizon for the limit. The data will, however, be for all types of weather from days on which no clouds were in the sky to days that were completely overcast.

### Materials and methods

Discontinuous hand readings are of little value in studying a problem of this sort. It was necessary therefore to obtain continuous simultaneous records from the different types of sampling units, and to check one against the other for accuracy.

An entirely new recording potentiometer of the duplex type was constructed for the work. The instrument was similar in many respects to the one exhibited at Pittsburgh in 1934 and identical to the one exhibited at St. Louis in 1935, except that the new instrument was a double one with two completely separate though identical recorders built into one cabinet and writing their respective records on one drum. Full details and instructions for the building and operation of this instrument can be found in the preceding issue of this journal (WALLACE, 3). Suffice it to say here, however, that this instrument is ideally adapted to the recording of light, due to several specific characteristics.

In the first place the bridge adjustment is practically universal, in that any millivoltage from 30 to 1500 can be obtained by the turn of a dial. This enables one to set up any given light intensity so that it will occupy the entire recording drum or any portion thereof. Also there is a universal control of the zero point of the bridge, so that one can place it at any point between the two ends of the drum. Therefore, when one uses a duplex recorder of this type, he can set up any two photounits for equal centimeter deflection irrespective of the actual millivoltage outputs of the units themselves. Also because of the zero adjustment he can set the two units so that their base lines

coincide on the drum, or place them 1 cm. apart or in any other combination that may be desirable. All curves given in this paper were obtained on this instrument.

Most of the data given below were obtained during the period from April 16 to May 6, 1936. The light station for all of the tests was two adjacent ventilators 4 feet high located about 15 feet apart on the roof of the laboratory. This position completely avoided shading of the photounits at any time. No adjustment whatever, except in figure 5, was made during the progress of a test. All tests on each combination of photounits were run for at least three days in succession, or until the type of curve could be predicted. All figures given in this paper are unretouched photographs of the records themselves. No actual foot-candle values for the curves are given, since it was felt that one is chiefly interested in the exact comparative values shown by the two units and that it was best not to complicate it.

The photocells used throughout these tests were the Weston photronic. The photocells were never exposed to the direct rays of the sun except on the occasions mentioned in the paper. As will be seen below, it is very necessary to take this precaution to avoid damage to the cells. The spheres mentioned in the paper were the common type of opalescent shades obtainable at any good electrical store and had transmission factors of 48 and 52 per cent. respectively. Two sizes were used, the small 8-inch size for the one-celled photounits, and the 12-inch size for the multiple-celled photounits.<sup>2</sup>

All photocell units were attached to rubber-covered wires which led down over the roof to the laboratory below where the recorder was in operation. Each photounit was likewise drained with a 10-ohm resistance placed directly across the lead wires at the input terminals of the recorder. This was to insure linearity between the current output of the cells and the light intensity.

The usual method of setting up the instrument for the different combinations was as follows. If possible each new series involving a new photounit combination was begun about noontime on a clear day. This enabled one to set the two units for identical centimeter deflection on the recorder drum. Setting up units for the first time in the evening or morning is simply guesswork and almost invariably results in lost time. If, however, they are set for equal deflection at normal or almost normal incidence at noontime, not uncommonly the records for the first day are just as perfect as any subsequent ones.

<sup>2</sup> The spheres can be the size best suited to your convenience since the sampling of radiation is independent of the size of the sphere used. Thus a series of determinations of a certain light intensity with one photocell under spheres of 5, 6, 7, 8, 10, and 12 inches respectively gave identical values when one corrected for the difference in transmission of the different spheres. The globes must be the white opalescent type and not those made of ground glass. One of the latter type was tested and found to give an error of 500 per cent. in the same test (series 7 below) in which the opal sphere gave a 5 per cent. error.



The setting of the zeros was adapted to the test series being run. It was sometimes not possible to forecast just which zero combination would best show the agreement or lack of agreement of the different photounit combinations. It was necessary therefore in these cases to set up the more desirable zero combination after one day's record was obtained. The zero combination found to be best suited to most of the photounit combinations was that in which one zero was at the very base of the drum and the other 1 cm. above it. It must also be remembered that the time axis for the two curves is not the same. The upper curve is always 5 minutes behind the lower curve. One must bear this in mind, since the exact centimeter deflection of any point on a record is the vertical distance of that point above the zero line for that curve. For most purposes, however, the distance between the same points of the two similar curves will give the agreement or lack of agreement between the two curves with sufficient accuracy.

### Experimentation

#### TYPES OF PHOTOCCELL COMBINATIONS

The more significant physical arrangements or orientations of the sensitive surfaces of the photocell were tested in these experiments. The ideal shape of the sensitive surface, if perfect sampling of radiation is to be obtained, would seem to be that of a sphere. This unit would sample the radiation falling on a point, and consequently be independent of angle. However, at the present time there are no photocells made with spherical receiving surfaces and possibly there may never be because of physical difficulties involved, and consequently this ideal cannot be reached.

The next most perfect sampling device would seem to be a unit in which a great number of sensitive surfaces of equal value and size would face in all possible directions. This condition is likewise very difficult or impossible to attain.

**TYPE A.**—A compromise was made with these conditions in the following experiments by placing a photocell on each of the six sides of a wooden cube and supporting this on short wire legs so that the center of the wooden cube was about 6 inches above a base support. A spherical shade, 1 foot in diameter, was then placed over the unit so that the center of this globe and the center of the photounit built around the wooden cube would coincide. We have in this combination a six-faced-sampling unit in the center of a large integrating sphere. Physically this unit should very adequately integrate the light falling on a point, since its sensitive surfaces face in all directions and the integrating sphere will tend to equalize the light and reduce errors due to unequal sensitivity of the individual photocells. The same unit without the integrating sphere was tested later and found to be quite inferior to the unit with the sphere, as described below. This six-celled unit under the inte-

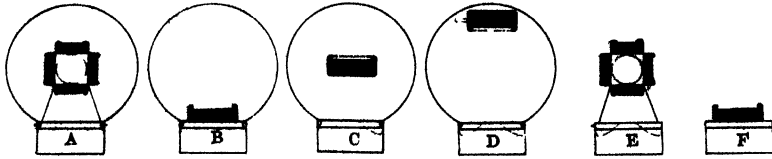


FIG. 1. Schematic diagram showing the arrangement of the different types of photounits: A. Six photocells fastened to the six sides of a cube and thus facing the four cardinal points, zenith, and downward, and enclosed under an integrating sphere; B. One photocell facing up from the base of an integrating sphere; C. One photocell facing up from the middle of an integrating sphere; D. One photocell facing downward from the top of an integrating sphere toward a reflecting surface in its base; E. A six-celled photo unit similar to A, except used with no sphere; F. A one-celled unit similar to B, except used without a sphere.

grating sphere is the one against which all other combinations were checked (fig. 1, A).

TYPE B.—A second type of photounit consisted of a single photocell facing up from the base of an opal diffusing-sphere. The sampling characteristics of this unit will be determined almost completely by the integrating characteristics of the globe itself, that is, the accuracy of the physical summation of the radiation falling on a point (fig. 1, B).

TYPE C.—A third type was a single photocell facing up from the center of an opal diffusing sphere. The sampling characteristics of this combination will depend to some extent on the photocell being up in the center of the sphere instead of at the base of the sphere, but to a greater extent in the integrating properties of the sphere itself (fig. 1, C).

TYPE D.—A fourth type consisted of one photocell fastened in the top of a sphere and facing downward toward a white, reflecting surface in the base of the sphere (fig. 1, D).

TYPE E.—A fifth type was the six-celled unit mentioned as type A but used without an integrating sphere, so that the cells were exposed to the direct rays of the sun (fig. 1, E).

TYPE F.—A sixth type consisted of one photocell mounted horizontally and facing the zenith and not covered by a diffusing sphere. This is the method commonly used by the Weather Bureau for sampling total radiation by means of pyrheliometers (fig. 1, F).

#### EXPERIMENTS ON PHOTUNIT TYPES

A series of tests was run for each of the types of photounits given above, and on certain other factors that affect the accuracy of sampling. As mentioned above, the light stations used in the tests can be considered as identical. I shall now survey the results of these tests.

SERIES 1. CAN IDENTICAL RECORDS BE OBTAINED.—The experiments of series 1 were designed to test all factors entering into the sampling of

light; the adequacy of the recorder, the freedom from stray electrical pick-ups, the use of identical photounits, the shape of the integrating shade, and any other factors which might prevent identical curves being obtained from what seemed to be identical sets of conditions. It was felt that identical records from two entirely independent but identical photounits, measuring the same light station at the same time on two entirely separate electrical circuits, connected to two entirely separate recording mechanisms writing on the same drum, would indicate that all factors entering into the tests were satisfactorily controlled.

This experiment was first run with type B photounits—that is, one photocell mounted in the base of each shade and facing zenith (fig. 1, B). The shades used were, however, not spheres as was originally planned since they were not available at the moment. Two other opalescent shades such as are shown in figure 2, A were substituted temporarily. The units were mounted

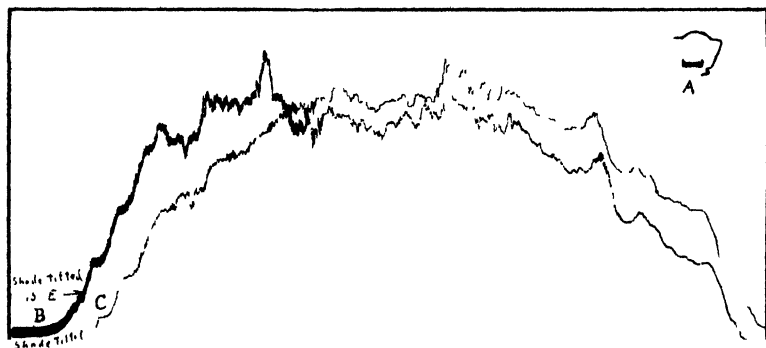


FIG. 2. Records for April 9, 1936, showing the effect of shape of integrating shade on the sampling of light. A. Shape of shade used; B. Curve for unit number one tilted 15 degrees to the east; C. Curve for unit number two tilted 15 degrees to the west. See text for further description.

on the ventilators and the bridges of the recorder set for equal centimeter deflections from zeros 1 cm. apart.

At the end of 3 days it was obvious that the two units were not sampling alike. Nevertheless, the discrepancy between the two records was consistent and duplicated each day so that it was possible to predict what the disagreement would be. After examining the duplicate records for a 3-day period it seemed obvious to me that the shade on unit number one was tilted to the east. Investigation revealed that not only was the shade on unit number one tilted 15° to the east but also that the shade on unit number two was tilted 15° to the west. The actual relationship of the two curves for the third day of the test is shown in figure 2. It will be noted that the two curves tend to diverge during the forenoon, run along fairly well together during the mid-

day period, and converge during the afternoon. Reference to figure 2, A, showing the straight-sided shades used as integrating units, will show that if unit one is tilted to the east and unit two tilted to the west the discrepancy found between the two curves in figure 2 would occur.

It is obvious from these results that it is not safe to use diffusing shades that are other than spherical in shape. The variation that showed up here, due to the two units not being level, proves there is unequal sampling of the light. Subsequent tests have shown, on the other hand, that little or no care need be exercised, even to the leveling of photounits, under spherical shades.

The test was repeated the following day with spherical shades. The curves obtained are shown in figure 3. It is almost impossible to find devia-

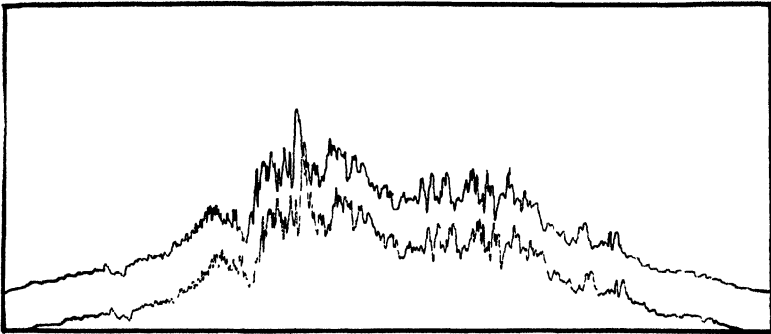


FIG. 3. Records for April 10, showing identical curves when spherical integrating shades were substituted for the type A of figure 2.

tions on the extreme points of more than 1 or 2 per cent., while the major portion of the curves is identical. It seems obvious from these data that the factor that had prevented identical curves in figure 2 is the type of shade used. One can answer therefore that identical records can be obtained from two identical photounits under the set of experimental conditions mentioned above. We are ready, therefore, to test the actual differences between the sampling from different types of photounits.

**SERIES 2. SIX-CELLED UNIT *vs.* ONE-CELLED UNIT, TYPE B, BOTH UNDER DIFFUSING SPHERES.**—This series of experiments was run to test type A photounit—the six-celled photounit with one cell facing each of the four cardinal points, zenith, and downward, against type B photounit—a single cell at the base of a sphere facing up. Figure 4 shows the curves from this combination of photounits. The upper curve is the six-celled unit, the lower one the one-celled unit. The zero for the six-celled unit curve is 1 cm. above that for the one-celled unit. It will be noted that during the later forenoon there is a period of about two hours with full sun (smooth part of the curve), followed by another two-hour period during which the light intensity is considerably

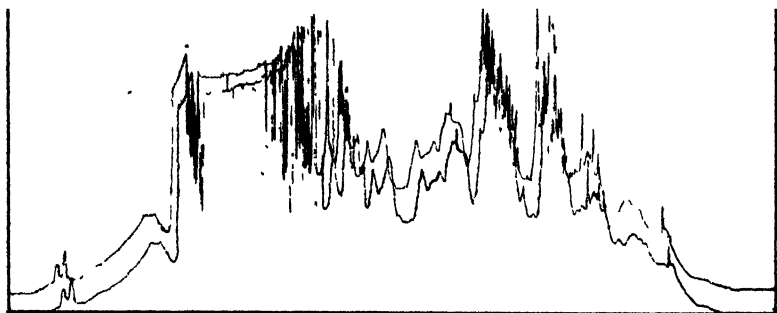


FIG. 4. Records for May 2, showing curves for type A and type B units in sampling of light. The upper curve, the type A or six-celled unit; the lower curve, type B or one-celled unit. Light conditions variable from heavy fog in the early forenoon to full sunshine just before noon, and variable light and cloud for the afternoon.

greater than that for the preceding period of full sun with no clouds. This increase of light intensity above that for full sun with no clouds is very common when there are large white clouds, and often appears on the records as close-spaced vertical lines such as are observed here. For the next 4 or 5 hours the sky was heavily overcast with no direct sun at all, followed by two periods when the sun again broke through for a short time to be followed by another period of completely overcast sky until dark. It is not necessary to interpret which curve is correct since the curves are almost identical. The maximum error of the extremes is about 5 per cent., while the major portion of the two curves is identical.

In figure 5 are shown the same two units on a very heavily overcast day. In this case the zero for the two curves is the same base line and the curves show up as parallel tracings with the curve for the six-celled unit 5 minutes back of that of the one-celled unit. The two curves do not agree well during



FIG. 5. Records for May 4, showing the sampling from the same combination as in figure 4, on a very heavily overcast, foggy day with no direct sun at all. The six-celled unit is the one lagging back of the other curve. The two units were set for equal amplitudes at point a; the lack of agreement prior to this point should therefore be ignored.

a part of the forenoon owing to the two units being set for unequal amplitudes. These were set equal at point *a*, and the agreement from here on is very close indeed.

The light conditions covered in figures 4 and 5 are for all extremes from full sunlight, through the marked fluctuations characteristic of alternate sun and shade with large white clouds, on to very heavily overcast sky with no sun at all. Throughout this great range of conditions the sampling of the two units is practically identical, the variations of the extremes being no more than 5 per cent. It would seem therefore that for all purposes, except those necessitating extreme accuracy, a type B photounit consisting of a single photocell facing up from the base of a sphere is just as adequate as the six-celled unit. This is very fortunate because the multiple-celled unit is very awkward to carry and difficult to use under field conditions where there is brush or other obstruction.

**SERIES 3. SIX-CELLED UNIT *vs.* ONE-CELLED UNIT, TYPE C, BOTH UNDER SPHERES.**—The difference between this series and the one just preceding is that in this case the one-celled unit is of type C, in which the single cell is placed up in the middle of the diffusing sphere facing upward, instead of in the base of the sphere facing up. The agreement between the two curves was good but somewhat inferior to that found in series 2 above. The maximum error of the extremes being about 15 per cent. as compared to the 5 per cent. in the other case. There is really no advantage whatever in this combination over the one cell in the base of the sphere facing up, so no further consideration was given it.

**SERIES 4. SIX-CELLED UNIT *vs.* ONE-CELLED UNIT, TYPE D, BOTH UNDER DIFFUSING SPHERES.**—In this series the one-celled unit was type D with the one-cell

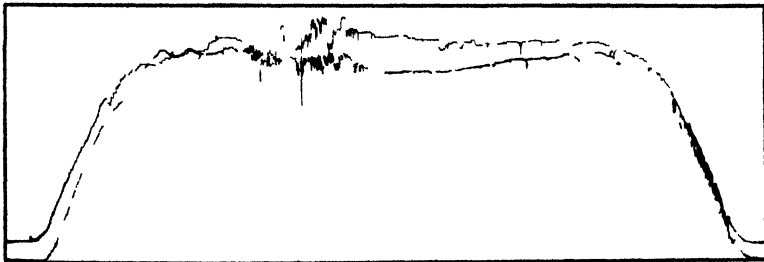


FIG. 6. Records for April 22, showing the sampling characteristics of type A photo unit and type D unit, the one cell in the top of a sphere facing downward. Upper curve, the six-celled unit; the lower curve, the one-celled unit. The day was completely clear except for a few small clouds just before noon. The two rather conspicuous dips in the curves just prior to the period of small clouds were due to a high haze, scarcely visible to the eye, which passed over at that time. This haze was quite visible when viewed with amber glasses.

fastened in the top of the sphere facing a reflecting surface in the base of the sphere. This combination gives a fairly good agreement for the early forenoon and the late afternoon as can be seen in figure 6. Then there is a period during both the forenoon and the afternoon when the curve (the one with the lower base line) goes about 15 per cent. too high. This can be readily seen in figure 6 if one raises the lower curve up so its base line coincides with the base line for the six-celled unit. This period of excessive height gradually gives way to normal and then becomes lower and lower and reaches its point of maximum divergence from the six-celled-unit curve at noon. The curve, in other words, is badly "sway-backed" during the middle of the day.

This is just the type of curve that would be expected from type 4 unit as can be easily seen by examining figure 1, D. During the early forenoon and late afternoon when the sun is not high a greater amount of the light hitting the sphere will reach the photocell. Then during the middle of the day the maximum light will be hitting that portion of the diffusing sphere against which the back of the photocell is mounted. This blind spot will naturally cause the curve to be low for that period. All subsequent tests have shown this fundamental defect in the curves for this unit. This type D unit is not to be recommended.

**SERIES 5. SIX-CELLED UNIT UNDER SPHERE *vs.* ONE-CELLED UNIT, TYPE F, WITHOUT DIFFUSING SPHERE.**—This method of exposing the sensitive surface of the receiving unit horizontally to and unprotected from the direct rays of the sun is the standard method in use by the Weather Bureau for the determination of total radiation by means of pyrliometers. It is theoretically possible for one to correct such records for normal incidence of the sun, that is, right angle or noonday incidence. One can do so, however, only when optical conditions are perfect, for only then does Lambert's cosine law apply. With the photocells this condition is only approximated, the disagreement being as much as 30 per cent. for low angles of incidence according to the technical data given by Weston for their cells.

Figure 7 shows the type of agreement between these two methods of sampling. It will be noted that the two curves are set on the same base line and have equal deflection at midday. During the forenoon the records show alternate sun and clouds and very poor agreement between the curves. There is a general tendency for the lower curve of the horizontally exposed cell to follow the cosine law but there are marked variations from it as will be noted by comparing points *a* and *a*<sub>1</sub> and other pairs of points in the same general region. Then the two curves approach each other rather closely at *b*. It is to be observed here, however, that the amplitude of the change of the six-celled curve at *b* is much less than it is for the uncovered cell. In the region of point *c* the situation is worse, since the curves now cross each other. At *d* the six-celled unit curve rises strongly while no such

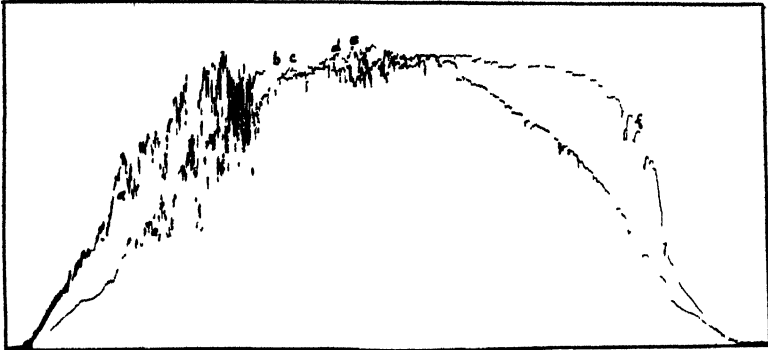


FIG. 7. Records for April 27, showing the sampling by type A unit and type F unit. The latter type is a photocell mounted horizontally and exposed directly to the rays of the sun. The upper curve, the six-celled unit; the lower curve, the one-celled unit. The forenoon was alternate light and cloud; the midday period slightly cloudy; and the afternoon almost completely clear. For further details see the text.

rise is evidenced by the curve for the other unit. The extreme situation is reached, however, at point *e* where the two curves are actually mirror images of each other. In the afternoon period, which was entirely clear except for a few scattered clouds, the true divergence of the two curves is plainly shown. It will be noted even here, however, in the region of *f* that light changes may be picked up by the six-celled unit which have little or no effect on the other unit.

Is it possible to correct the single-celled unit record to that for the six-celled unit with any degree of accuracy? Theoretically this can be done, as has been mentioned. That is, if there had been no clouds or haze at all during the afternoon and if the photocell obeyed the cosine law exactly, the correction would raise the lower curve up to match the upper curve. Since, however, there were clouds and also since the photocell obeys the cosine law only fairly well, such a correction is of little value. Moreover corrections for the horizontally mounted cell involve laborious calculations before the records can be used.

Much more important, however, than the failure of the photocell to obey the cosine law, or the work involved in the correction of the curves, is the fact that the six-celled unit and the horizontally mounted naked unit do not sample radiation alike even during midday when sun incidence is normal. This indicates a fundamental defect for which there is no correction. Referring again to points *b*, *c*, *d*, and *e*, it will be recalled that all possible combinations from difference in amplitude for a given light variation to a complete reversal or mirror image for the two curves is shown. This midday discrepancy is due to the presence of large white clouds. It has already been mentioned that very frequently large white clouds will increase the light



intensity far above that for a perfectly clear day. These clouds, depending upon their position in the sky, may affect the two sampling units differently. If they are in a low-lying bank they will record in full on the six-celled unit and yet may fail to register at all on the horizontally mounted unit. This has often been observed in such instances as that indicated by points *b*, *c*, *d*, and *e*. Again, one large cloud passing between the unit and the sun must affect the naked cell very strongly but may not greatly affect the other unit. In case the rest of the sky is free of clouds, the drop in the curves for the two records may be quite similar, but in case several other highly reflecting clouds are along the horizon then this record may show no drop at all since points of higher intensity counteract points of lower intensity. This physical situation can be well illustrated by the contrast in sharpness of shadows on a perfectly clear day with the same shadows on a day with white-haze and highly reflecting clouds. The shadows in the latter case are much less dense due to the reduced relative importance of the direct rays of the sun. If then one is to apply Lambert's cosine law with any degree of accuracy to records of this sort, he must correct not only for the direct rays of the sun but also know the angle, duration, and intensity effects given by each cloud of importance in the sky and correct for them. This is, of course, impossible and it is just as impossible to unscramble records of this type.

Another point that must be brought out is that when the day is overcast enough it is immaterial whether integrating spheres are used or not. Thus

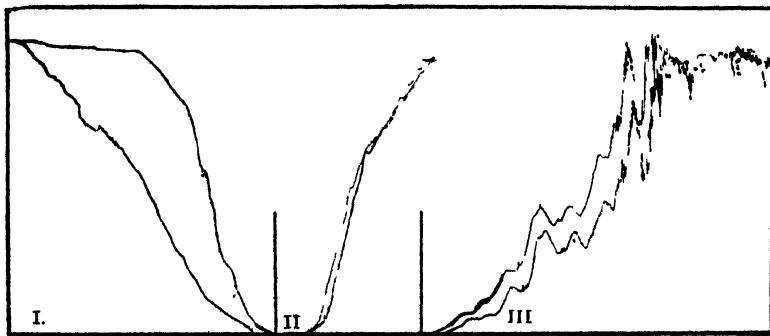


FIG. 8. Records giving a summary of the sampling by type A and type F units as shown on three successive days. I. Records for the afternoon of April 28, showing the divergence of the two curves as expected from the cosine law on a perfectly clear afternoon. II. Records for the forenoon of the day following, April 29, showing the agreement between type A sampling and type B. Type F was converted into type B merely by placing the sphere over it. III. Records for the forenoon of the following day, showing the sampling for type A and type D units. Part of this forenoon was heavily overcast, so the two units sample almost exactly alike (the two units are not set for identical amplitudes which causes the curves to drift apart, but this does not count. When, however, the direct sun breaks through along the flat part of the curve later, the sampling is similar to that shown at *b*, *c*, *d*, and *e* of figure 7.

in III of figure 8, we have the six-celled unit with its sphere and the horizontally mounted single cell with none. This pair of records has been plotted on the same base line but unfortunately were not set for equal amplitudes. If one allows for this difference in setting then the very close agreement between the two units becomes obvious. The uniformly overcast sky has now become the integrating unit and consequently the records agree. Notice how quickly, however, discrepancies appear when the sun breaks through at the top of the curves; the same situation as that along points *b*, *c*, *d*, and *e* of figure 7.

Another interesting point is presented in figure 8, I, which shows the typical curves for the naked photocell and the six-celled covered unit on a clear afternoon. Then figure 8, II, shows exactly the same conditions for the next forenoon, except that both units are now covered. The addition of the sphere brings the two units into perfect agreement on sampling, in the same manner as shown earlier in text figures 4 and 5.

**SERIES 6. SIX-CELLED UNIT WITHOUT INTEGRATING SPHERE, TYPE E vs. ONE-CELLED UNIT WITH SPHERE.**—In this combination, the situation was just reversed from that in the preceding test. The one-celled unit was now under an integrating sphere while the six-celled unit was not. In this combination the agreement is fair but the variations were as much as 15 per cent. This discrepancy is undoubtedly due, in great part, to the unequal sensitivity of the six photocells. That is, in case the strongest radiation is coming from the direction where the most sensitive cell intercepts it the curve will be high or vice versa in case a weak cell is in that position. Also the sampling of each passing cloud will be too high or too low depending on the photocell intercepting the light. The integrating sphere would have smoothed out most of this. In this combination also the curves become identical when the sky is heavily overcast. Another serious objection to this combination is the probable damage to the cells from exposure. This latter point is taken up in greater detail below.

**SERIES 7. PHYSICAL CHARACTERISTICS OF PHOTOUNITS A AND B.**—After the experiments reported above were complete, it seemed desirable to determine the physical characteristics of the two most dependable types of units. The six-celled unit (A) and the one-celled unit (B) were therefore tested in the darkroom. One of these tests was to place the unit on a turntable at a given distance from a 1000-watt Mazda bulb and make a reading. The table was then turned 90° and the next quarter tested and so on until all four were compared. The unit was then placed on a tilting device that enabled one to expose the unit to light from any angle without modifying the distance from the light.

In table I are given the data for the six-celled unit. The first set of tests

**TABLE I**  
**PHYSICAL CHARACTERISTICS OF TYPE A PHOTUNIT\***

CELL NO.	READING WHEN FACING LIGHT	
	WITHOUT SPHERE	WITH SPHERE
1 .....	89.0	94.8
2 .....	89.1	94.8
3 .....	95.6	97.4
4 .....	100.0	100.0
5 .....	89.0	97.0

\* The unit was revolved around its vertical axis to give the readings for cells 1, 2, 3, and 4 and turned on its side for cell 5 which was the cell that normally faced up. All cells are expressed in terms of the strongest one, number 4.

was made without the integrating shade. It will be observed that when the successive cells faced the light the readings differed, owing to the unequal sensitivity of the cells. When the shade was put on these, differences in sensitivity dropped to about one-half their former value owing to the integration of the globe. When the unit was tilted the reading obtained with cell 5 facing the light was about the average given for the four preceding cells. This is what would be expected from the combination. The type A photounit is shown by these data to have an error of about 5 per cent. when predominantly unilateral light is directed in succession toward the four photocells. This variation is only half as great as that for the four photocells exposed without the sphere. With matched cells this error should disappear.

When the unit with a single cell facing up from the base of a sphere was revolved about its vertical axis no variation of more than 0.1 per cent. was found. When the same unit was tilted through an angle of  $180^\circ$ , 10 feet in front of a 1000-watt Mazda bulb the maximum variation found was 5 per cent. On closer testing this variation was found to occur only when the angle of incidence was  $18^\circ$  or less. In other words, after the unit was tilted  $18^\circ$  toward the source of light the reading would remain constant for the rest of the  $144^\circ$  arc, or until within  $18^\circ$  of complete inversion of the unit. Even within the  $18^\circ$  angle where the variation occurred the error was merely progressive, first showing up at  $18^\circ$  and reaching 5 per cent. at  $0^\circ$ . This  $18^\circ$  portion would represent approximately 1 hour at sunrise and 1 hour at sunset when the record might show as much as a 5 per cent. error. For the rest of the day, no error of consequence would occur. This sampling would seem to be adequate for all except the most exacting problems.

**SERIES 8. DEPENDABILITY OF PHOTOCELLS.**—During the progress of these studies several points of importance to those working with photocells were

encountered. The first and perhaps the most important is the detrimental effect of direct sunlight on the Weston cell. This trouble first appeared in some further tests of type E photounit in which the cell is exposed directly to the full rays of the sun. This cell after several days of hot clear weather became completely erratic. Within a few minutes after it had even been set for equal deflection with the other unit it would be off-scale or have dropped to half of its former value. It was reset seven times in a period of 4 hours in an unsuccessful attempt to keep it on scale. It was then allowed to run for several days, set for only a small part of the full scale of the bridge. It immediately showed the following effects. During the early morning and late evening it usually gave a curve which agreed very well with the other unit but just as soon as the temperature reached a certain value the curve became erratic. The cell was then laid aside as being defective.

The next appearance of this trouble was during the last days that series 6, described above, was run. In this series the six-celled unit was tested without the diffusing sphere. At first good agreement in curves between this unit and the unit under the sphere was obtained, but suddenly the six-celled unit became very erratic. The results are shown in figure 9. The upper

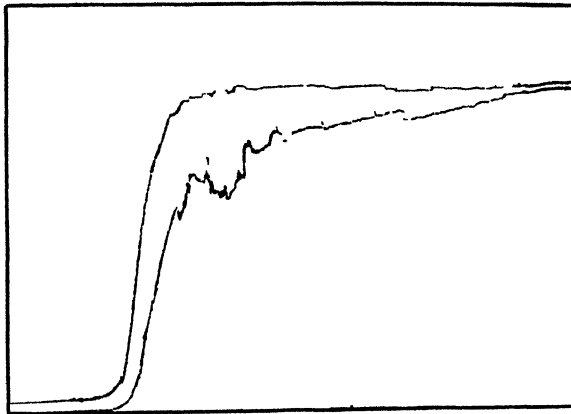


FIG. 9. Records for May 16, showing defective sampling by type A, the lower curve, as against type B the upper curve. These two types had always given agreement before. The type A unit had, however, been exposed for several days directly to the rays of the sun without the protecting sphere. It was possible from this curve to forecast that the photocell facing east had been badly damaged. See text for full description.

curve is for unit B and the lower for unit A. It will be observed that the two units start off in the early morning with very close agreement between the curves. But, as mentioned above, the curve suddenly becomes erratic when a certain light or temperature level is reached. The curve then slowly climbs and, as other cells of the unit receive the light, finally reaches the

value it should about noon. For the rest of the day (not shown in the figure) the curves agreed quite closely.

On the basis of this it was obvious that something was wrong with the photocell facing east and possibly the one facing south, but that the ones facing zenith and west were all right. The unit was removed from the roof and taken into the darkroom for recalibration of the individual photocells. This check-up, on May 28, was almost exactly one month later than the last calibration of the cells, on April 27. The results of this check-up are shown in table II. The cell facing east had dropped to 17.3 per cent. of its April

TABLE II  
CALIBRATION OF PHOTOCELLS\*

DATE	NUMBER OF CELL					
	1 (EAST)	2 (SOUTH)	3 (NORTH)	4 (WEST)	5 (ZENITH)	6 (DOWN)
April 27	100.0	85.7	100.0	80.0	80.0	80.0
May 28	17.3	41.7	80.0	80.0	80.0	80.0
May 28 Cells wedged	100.0	85.0	100.0	unchanged	unchanged	unchanged

\* Sensitivity expressed in terms of cell 1 as 100.

27 value. The one facing south had dropped to 41.7 per cent. The one facing north was 80 per cent. and the remaining three were apparently normal. The method of checking these values was to expose the cells in succession to a 1000-watt bulb at a distance of about 18 inches, which gave about 1000 foot-candles. It was then assumed that those cells retaining their relative sensitivity were unchanged. Subsequent tests have borne out these assumptions. If the tests are run rapidly to compensate for voltage fluctuations and repeated several times, the results are accurate to within 5 per cent., which is adequate for comparisons of this sort.

When it was found that one cell had dropped to 17.3 per cent., in terms of the value of the three unchanged cells, it seemed reasonable that the trouble might be due to loose contacts within the cell case since the glass face itself was quite loose. To test this three small match-stem wedges were slipped between the glass face and the composition case. The cell immediately came back to full calibration value and remained there (table II). The same procedure on the other two cells that were defective brought them back to full value. Similar treatment of the three normal cells failed to change their sensitivities.

It was now obvious that something was defective in the contact system within the case. The poorest cell was then placed on its back in a crucible full of acetone for an hour to dissolve the cement and allow the back to un-

screw. These cells are made up of a metal disc on one side of which a metallic material is deposited. The back of the disc is one electrode and the front the other. On the front of the cell between the glass face and the face of the disc is the front collector or electrode-ring contact. The fingers of this ring are visible around the edge of the face. Another ring just like this one fits against the back of the photocell disc. The composition back is supposed to screw down and hold these two rings in contact with the disc. The rings are, however, very thin and have, at most, not more than 0.5 mm. to allow for contraction and expansion. Apparently when the cell is exposed directly to the rays of the sun the case is stretched by the pressure developed by the heat and on cooling part of the stretch remains. As a result the cells may become erratic on exposure to the sun.

It seemed reasonable that if suitable constant-pressure springs were utilized to maintain the electrode contacts, these defective cells might be fixed. To accomplish this a small hole was drilled in the exact center of the screw-back of the cell. A clock spring was cut into short lengths, two of which were fitted at right angles across each other with a hole drilled in the middle of them.<sup>3</sup> These were mounted on the screw-back with a small bolt. A thin bakelite disc, the diameter of the photocell disc, was placed against the back electrode ring for the springs mechanism to push against. This disc was necessary to avoid damage to the photocell. Part of the flange of the screw-back which was formerly supposed to hold the electrodes in place was cut off to give room for the spring legs to work. The unit was assembled by screwing the back in as usual. The first cell (the one that had dropped to 17 per cent. normal) worked perfectly so the two other defective ones were fixed. The three then had the same sensitivities that they had on April 27. The other three normal cells were now rebuilt with like results. If changes in sensitivity occurred they are less than 5 per cent. and cannot be detected with the test method used. These six cells have now been in use for more than two months and have shown complete dependability. I have, so far, found no evidence that these particular Weston photocell discs were damaged by the direct exposure to the sun that they received. I am able to report, however, that certain electrocells, tested in conjunction with C. I. GUNNESS of Massachusetts State College, have shown that excessive exposure to sun has damaged the photocell discs. It seems highly inadvisable therefore to expose cells of this type to direct sunlight.

Through the courtesy of B. E. GILBERT of Rhode Island State College, I am able to report the results of tests, similar to those on dependability given above, which were run on some of his photocells. After finding the defective cells in my own unit I thought it would be interesting to run a like test on his

<sup>3</sup> These springs must not exert more than about a pound pressure for otherwise they may short the photocell disc.

multiple-celled photounit. These cells had never been exposed to direct sunlight. When the five cells were tested, however, one was found to be only 25 per cent. as strong as the other four. When it was wedged, it became the best cell of the five in the unit. Also one of the large Weston illuminometers used in the same laboratory increased its reading from 940 foot-candles for this same light to 1500 foot-candles. This occurred in response to pushing the glass face of the cell back and forth several times. No subsequent wedging or moving of the face would change the reading in the least. This latter effect has been observed very often during the progress of these studies. Perhaps this may be due to some oxidation which reduces the efficiency of the electrode contacts. There are other photocells of the voltaic type which seem to have few if any of the mechanical weaknesses which have been described above. One of these, an electrocell, has received more than two months' exposure under a sphere and has maintained perfect stability.

In one case, during these experiments, lightning struck nearby and burned out a Weston photocell. On examination, a tiny pit, about  $\frac{1}{4}$  mm. across and about the same depth was observed on the face of the photocell disc. The surface of the disc was explored by laying the disc on one copper wire and touching a moistened wire to various points on the face. The pit already mentioned was the dead spot. A tiny bit of wax (half beeswax and half rosin) was put on the pit and a tiny bit of paper pushed down against it. The cell now functions perfectly and its calibration seems unchanged.

#### INTEGRATION OF LIGHT RECORDS FOR VARIOUS FOOT-CANDLE LEVELS

In view of the interest shown in a suggestion I made at the symposium I shall elaborate somewhat and give a figure to make the suggestion more concise and clear. It would seem from the data already available on the light intensities required for various processes, especially photosynthesis, that different species have different optima. If, then, one is working with a plant that reaches its maximum rate of photosynthesis at the 2000-foot-

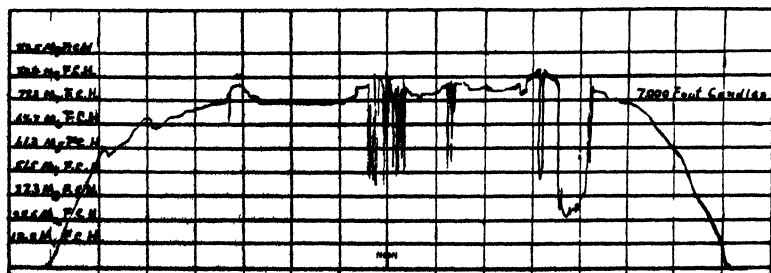


FIG. 10. Record for August 15, 1935, showing a method of integrating light records at various foot-candle levels for processes not requiring full sunlight intensity for their maximum rate. For full description see text.

candle level, then those portions of the light record lying above that level act only to obscure the true relationships. Might it therefore not be of value to integrate the records for various levels and give each investigator those values adapted to his needs?

In figure 10 is shown an ordinary record for Storrs, Connecticut, on August 15, 1935. It is calibrated in 1000-foot-candle levels. At the left of the figure are shown the integrated values obtained with a planimeter for these various plateaus. I have designated the first one as  $13.8 M_1$  f.c.h. This would read 13.8 foot-candle-hours of light below 1000-foot-candle level. The second level would be  $25.6 M_2$  f.c.h. or 25.6 foot-candle-hours below the 2000-foot-candle level, etc. It will be noted that each level is a complete summation of the total light quantity to that level so that finally when the level of maximum intensity for the day is reached the figure automatically becomes the total light quantity for the day. Thus the top plateau can be read at  $80.5 M_3$  f.c.h. or as 80.5 times  $M$  (1000) or 80,500 foot-candle-hours.

### Summary

1. A comprehensive series of experiments on various physical arrangements of the sensitive surfaces of photocells were run to determine the combinations which gave the most perfect sampling of light. These different sampling devices were connected to a duplex recorder so that the sampling of the units was recorded as continuous, simultaneous curves of the same light station on the one-recorder drum.

2. Six types of photounits were tested. Of these the two found to have the least error in sampling were a six-celled unit in which a photocell was fastened on each of the six sides of a cube and the whole enclosed beneath a spherical integrating shade of opal glass, and a one-celled unit in which one photocell was placed in the base of an integrating sphere facing up. There was no significant difference between the sampling of these two units, but certain other considerations would indicate that the one-celled unit may be preferable.

3. The determination of visible radiation by exposure of the receiving unit horizontally to the direct rays of the sun, as is done by the Weather Bureau for total radiation, does not seem suited to physiological studies, owing to the impossibility of correcting by the cosine law for lateral light and other effects. Also the exposure of photocells to the direct rays of the sun may be very detrimental to them.

4. A suggestion is made for a method of integrating light records for various foot-candle levels to enable each investigator to choose those levels of significance in his studies.



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# A PRECISE METHOD, WITH DETAILED CALIBRATION FOR THE DETERMINATION OF ABSORPTION COEFFICIENTS; THE QUANTITATIVE MEASUREMENT OF THE VISIBLE AND ULTRAVIOLET ABSORPTION SPECTRA OF ALPHA CAROTENE, BETA CAROTENE, AND LYCOPENE<sup>1</sup>

ELMER S. MILLER<sup>2</sup>

(WITH EIGHT FIGURES)

## Introduction

Owing to recent developments in amplifying tubes and circuits, it has been possible to design a spectrophotoelectric apparatus which permits the precise measurements of the absorption coefficients of alpha carotene, beta carotene, and lycopene. The absorption curves are important as: (a) *a criterion in following the isolation of plant pigments* (6), and (b) *standards in analytical methods*.

Recently ZSCHEILE (11) and MILLER (7) have shown that the spectrophotoelectric method satisfactorily solves the problem of pigment differentiation. This method makes possible not only the determination of the total differentiation for each group (chlorophylls, carotenes, and xanthophylls), but also the analysis for the total of each component within the respective groups. Because no separation of pigments into groups or components within a group is necessary, the spectrophotoelectric method is rapid.

This paper presents a detailed calibration of the apparatus and a comprehensive discussion of the limitations of the method in conjunction with the quantitative absorption spectra of alpha carotene, beta carotene, and lycopene in the visible and ultraviolet region.

## Nomenclature

The nomenclature employed in quantitative spectroscopy is somewhat confusing to biologists because different units and symbols are employed to express absorption. In this paper alpha ( $\alpha$ ) in equation 4 is plotted as the ordinate with wave lengths expressed in Ångstrom units as the abscissa.

The quantitative absorption spectrum of an organic compound is determined in the following manner. In a homogeneous medium, according to Lambert's law, the amount of radiant energy absorbed is expressed by equation 1.

$$I_x = I_0 \cdot 10^{-\beta x} \quad (1)$$

<sup>1</sup> Contributions from the Department of Botany, University of Minnesota.

<sup>2</sup> This research was started at the Herbert Jones Laboratory at the University of Chicago in 1932, and continued at the Department of Botany at the University of Minnesota from September, 1936. The absorption curves in the visible region for the mentioned carotenoids have been published in the *Botanical Gazette* and *Plant Physiology*.

When beta ( $\beta$ ) is proportional to concentration, Beer's law is obeyed, and

$$\beta = \alpha c. \quad (2)$$

Then

$$\log_{10} \frac{I_o}{I_x} = \alpha c x \quad (3)$$

or

$$\alpha = \log_{10} \frac{I_o}{I_x} / c x. \quad (4)$$

$I_o$  = intensity of light transmitted by the solvent cell.

$I_x$  = " " " " " " solution cell.

$x$  = thickness of absorption cell in centimeters.

$c$  = concentration in grams or moles per liter.

$\alpha$  = specific absorption coefficient for grams per liter, or molecular absorption coefficient for moles per liter.

McNICHOLAS (5) plots "absorbency" as the ordinate. He defines this symbol as:

$$\text{"absorbency"} = \log_{10} \frac{I_o}{I_x} - bcK \quad (5)^3$$

Since his units are the same as those employed in equation 4,  $K = \alpha$ . WEIGERT (10) employs  $E$  to express the molar absorption coefficient calculated as the logarithm to base 10. When the molecular weight is not known,  $\alpha$  is frequently calculated on the basis of a 1 per cent. concentration. In this paper, unless stated, the gm.-cm. units will be employed.

## Materials and methods

### SPECTROPHOTOELECTRIC APPARATUS

The design of the apparatus is similar to the one described by ZSCHEILE (12), save that certain modifications have been made so that narrower slit-widths could be employed.

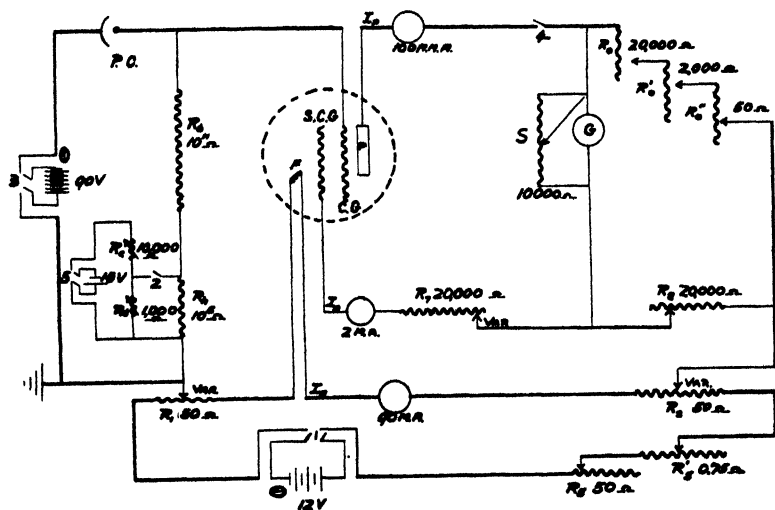
The spectral region is isolated from the continuous spectrum by a Zeiss fixed arm spectroscope (deviation  $90^\circ$ ), used as a monochromator. Referring to wave length 4861 Å, the effective aperture is  $F/6.4$  for the entire instrument. The error of the wave length drum is less than  $\pm 1$  Å at 2150 Å, and  $\pm 3$  Å at 7800 Å. Although the Zeiss instrument is equipped with achromatic lenses, a slight adjustment is necessary to correct for chromatic errors.

For the visible region, a 50 c.p. automobile Mazda bulb connected to batteries is employed as the light source. For the ultraviolet region a Urey type of hydrogen discharge tube operating at 4000 volts and 0.8 amp. is rather satisfactory.<sup>4</sup>

<sup>3</sup>  $b$  = cell length in centimeters.

<sup>4</sup> The tube was lined with a 5-mm. quartz tube. This precaution and the operation

A DuBRIDGE and BROWN (7) circuit (fig. 1) is employed to measure the photoelectric current. In this circuit an F.P. 54 vacuum tube (General



**Fig. 1.** A modified DuBridge d.c. amplifying circuit.

Electric) is employed for the amplification of the photoelectric current. The photoelectric current is passed through a high resistance  $R_g$  ( $1 \times 10^{11}$  ohm), and the potential across this resistance is impressed on the grid of the amplification tube. The amplified current is measured by a galvanometer (Leeds Northrup Company: 2500-b, period 6 sec.). The sensitivity of the galvanometer is controlled by an Ayrton shunt. The photocell is a caesium oxide gas-filled type with internal and external grounded guard rings. The sensitivity curve of this cell has been described by YOUNG and PIERCE (13).

The entire apparatus was set up in an underground laboratory in which the temperature fluctuates less than  $\pm 0.4^{\circ}$  C. in 24 hours. With proper electromagnetic shielding, a stable voltage sensitivity of 225,000 mm. per volt and a current sensitivity of  $1 \times 10^{-16}$  amp. per mm. is attained. These sensitivities are for 4 meters scale-to-galvanometer distance and a galvanometer sensitivity of 0.6. The galvanometer drift is less than 0.5 cm. per hour with oscillations  $\pm 1$  mm. Usually 40 to 50 seconds are sufficient for the measurement of two absorption coefficients. The cell carriage, which holds three absorption cells (Hilger type 291), is placed behind slit no. 2. Thus from four transmissions, two absorption coefficients may be calculated.

The over-all error (difference between  $I_0'$  and  $I_0''$ ) for the apparatus, including fluctuations in light source, amplification tube, photoelectric cell,

at 0.8 amp. prolongs the life of the tube greatly. The present tube has been in operation over ten months. It has run 500 hours on one filling with hydrogen.

and galvanometer, was less than  $\pm 0.25$  per cent. for the visible region and less than  $\pm 0.35$  per cent. for the ultraviolet region. The larger error for the ultraviolet region was due to fluctuations in the light source. This error should not be confused with the total error involved in  $\alpha_T$ .

### CALIBRATION

The calibration of the apparatus is described in detail so as to familiarize other investigators with the method employed in obtaining these spectroscopic data.

The Cooper-Hewitt mercury vapor arc was employed for the calibration of the wave length drum and the collimator lens. Figure 2 contains two

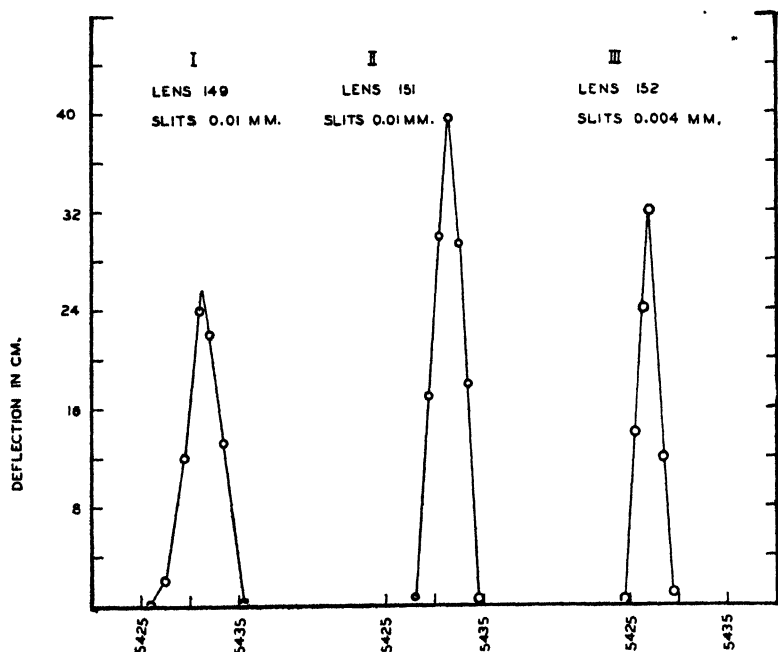


FIG. 2. Absorption curves of the 5460.7-Å mercury arc line.

absorption curves of the 5460.7-Å mercury line. After a preliminary attempt at setting the wave length drum and collimator lens, curve I was obtained. From this curve, with the wave length drum set at wave length 5432 Å, the collimator lens was again focused. These adjustments showed that the maximum galvanometer deflection was obtained with the collimator lens set at 150. Then curve I was repeated with readings taken at approximately one Angstrom intervals. These data are plotted in curve II. This procedure was again repeated and curve III was obtained. Thus, the 5460.7-Å line was in best focus with the collimator lens set at 150 and wave

length drum at 5432 Å. The agreement between curves I, II, and III is excellent, considering that the minimum error in the wave length drum setting is  $\pm 0.5$  Å. The maximum discrepancy between two wave length drum calibrations at six-month intervals for both the visible and the ultraviolet region was 2 Å.

The apparatus was tested to ascertain whether correct measurements could be obtained, according to the following procedure.

- A. Known voltages were impressed on the grid of the amplification tube and the deflections of the galvanometer were observed. Direct proportionality was found.
- B. The proportionality between the response of the galvanometer and the intensity of the incident light on the photoelectric cell was tested as follows:
  1. With the deflections of the galvanometer 80–100 cm. (reduced to 35 cm. at wave length 2200 Å) the response was measured when a series of screens was interposed. These data are summarized in table I.

TABLE I

COMPARISON OF SCREEN TRANSMISSIONS MEASURED BY THERMOPILE AND PHOTOELECTRIC APPARATUS WITH ABSORPTION CELLS IN PLACE

APPARATUS	SCREEN TRANSMISSIONS		
	SCREEN NO. 1	SCREEN NO. 2	SCREEN NO. 3
Thermopile . . . . .	73.4	54.8	32.7
2250 Å with cell no. { 1 . . . . .	73.0	54.6	33.0
{ 2 . . . . .	73.3	54.4	33.0
{ 3 . . . . .	73.5	54.4	33.1
3000 Å with cell no. { 1 . . . . .	73.0	54.6	32.5
{ 2 . . . . .	73.0	54.4	33.0
{ 3 . . . . .	73.3	54.4	32.9
3800 Å with cell no. { 1 . . . . .	73.5	55.0	32.9
{ 2 . . . . .	73.6	54.7	32.4
{ 3 . . . . .	73.3	55.0	32.7
4000 Å with cell no. { 1 . . . . .	73.8	54.4	32.4
{ 2 . . . . .	74.0	54.8	32.4
{ 3 . . . . .	74.0	54.3	32.2
5000 Å with cell no. { 1 . . . . .	73.6	54.4	32.4
{ 2 . . . . .	73.8	54.8	32.2
{ 3 . . . . .	73.4	54.8	32.3
6000 Å with cell no. { 1 . . . . .	73.7	54.5	32.3
{ 2 . . . . .	73.8	54.3	32.7
{ 3 . . . . .	73.7	54.3	33.0
7000 Å with cell no. { 1 . . . . .	73.6	54.6	33.1
{ 2 . . . . .	73.7	54.4	32.8
{ 3 . . . . .	73.7	54.4	33.0

Because three absorption cells were employed in this study, the screens were interposed with each of the respective cells in the path of the beam of light at 1000-Å intervals. Previously, a similar test was made with only one cell and screen no. 2 at 100-Å intervals. The transmission values of the screens were measured with a thermopile. Owing to variations in the respective position of the screens, the error in this experiment is 1 per cent.

2. The apparatus was tested for the linearity of response to incident light intensities which gave 30-, 60-, and 120-cm. deflections of the galvanometer, respectively. This was primarily to check "fatigue effects" in the photocell and "capacity effects" in the amplifying circuits. When the screens were interposed, constant transmission ratios at the different light intensities were obtained.

C. Standard solution.—As a check on the total error of the apparatus, light source,<sup>5</sup> and observer, the absorption coefficients were measured on a standard solution for the visible and ultraviolet region. This solution consisted of equimolar concentrations of potassium chromate and copper sulphate (pentahydrate) dissolved in a two-normal

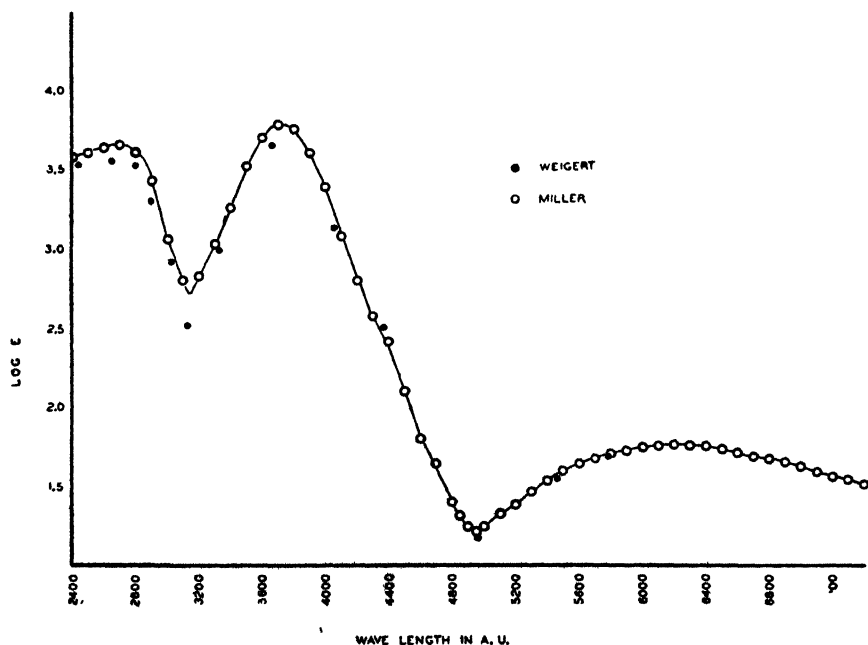


FIG. 3. Absorption spectra of equimolar concentrations of potassium chromate and copper sulphate (pentahydrate) in 2 N ammonia.

<sup>5</sup> Occasionally when the hydrogen discharge tube has been in service for some time, new bands are superimposed on the hydrogen lines. Some evidence has been obtained that the presence of these new bands may affect the value of the absorption coefficient.

ammonia solution. This standard was first employed by WEIGERT (10), VON HALBON (2), and recently by SMITH (9). The absorption spectrum of this standard is presented in figure 3. The dots and the circles represent the values obtained by WEIGERT (10) and the writer respectively. Considering the low sensitivity of WEIGERT's apparatus and the summation of the total errors in the two absorption curves, the agreement is about as good as may be expected. Unfortunately, this inorganic salt mixture has the following limitations as a standard:

1. The values of the absorption coefficients are affected by the normality of the ammonia solution.
  2. For the visible region, the absorption bands are too broad for this mixture to be a good standard in quantitative absorption spectroscopy. This will become apparent in the forthcoming discussion under the heading "Slit data and scattering of light."
- D. Calibrated glass filter.—Through the courtesy of the Bureau of Standards a calibrated filter was obtained. The transmission of this filter was measured with a thermopile at the wave lengths stated in table II.

TABLE II

COMPARISON OF TRANSMISSION VALUES OF GLASS FILTERS AS MEASURED BY THERMOPILE AND PHOTOELECTRIC APPARATUS

APPARATUS	TRANSMISSION VALUES		
	3020 Å	3130 Å	3340 Å
	%	%	%
Thermopile	29.5	54.5	86.0
Photoelectric	$28.9 \pm 0.2$	$54.6 \pm 0.2$	$85.9 \pm 0.1$

The photoelectric data agree within the experimental error with the transmission values obtained with the thermopile. This is an excellent filter to check on the total error because of its steep slope. If the prism setting is more than  $\pm 1.0$  Å in error, the transmission error is increased by a factor of two.

#### SLIT DATA AND SCATTERING OF LIGHT

Before comparisons are made with data obtained by investigators in different laboratories, *i.e.*, the use of the absorption curves in quantitative analyses, it is essential to state:

1. Slit-width in millimeters actually employed at the respective wave lengths.
2. The effective dispersion, *i.e.*, the slit-width in terms of Å per mm. of slit-width to permit the calculation of the spectral region isolated.

The slit data for the photoelectric apparatus is presented in table III.



TABLE III

SLIT DATA FOR ULTRAVIOLET REGION (HYDROGEN DISCHARGE TUBE AT 0.8 AMP.) AND VISIBLE REGION (50 C.P. MAZDA LAMP) WITH A 2-CM. QUARTZ CELL FILLED WITH ETHANOL

WAVE LENGTH	REGION ISOLATED $\text{\AA}/\text{mm.}$ OF SLIT-WIDTH	SLIT-WIDTH	SPECTRAL REGION ISOLATED IN $\text{\AA}$	DEFLECTION OF GALVANOMETER
$\lambda$	$\lambda/\text{mm.}$	$\text{mm.}$	$\lambda$	$\text{cm.}$
2200	65	0.20	13.0	19.5
2300	76	0.16	12.2	20.0
2450	102	0.08	8.2	69.1
2600	132	0.04	5.3	62.9
2900	204	0.025	5.1	76.0
3450	388	0.022	8.5	65.6
4000 (Quartz)	630	0.022	13.8	60.8
4000 (Flint)	160	0.025	4.0	49.4
4200	200	0.016	3.2	59.0
4500	240	0.007	1.7	99.0
4800	302	0.0033	1.0	90.7
6000	680	0.002	1.35	120.0
7200	1400	0.002	2.8	132.8

The limits of the wave lengths emitted from the second slit is two times the slit-widths due to overlapping of rectangles of light (finite spectral regions isolated) emitted from slit number II. Hence, the base of the frequency histogram of wave lengths is twice the respective slit-width. In this case the distribution curve is a triangle rather than one which approaches the normal curve. Therefore the population within the center half of the histogram (actual slit-width employed) includes 75 per cent. of the total wave lengths emitted. Data presented in table III show that in the visible and ultraviolet region, 75 per cent. of the total wave lengths emitted from the second slit are between 1.0 and 4.0  $\text{\AA}$ , and 5.1 and 13.8  $\text{\AA}$  for the visible and ultraviolet regions respectively.

SMITH (9) employed 0.07-mm. slit-widths which gave a spectral region 8.0  $\text{\AA}$  wide for the center half of the absorption curve of the 5460.7- $\text{\AA}$  line. This does not define the spectral region isolated because the width of the mercury line is not only affected by the slit-widths but also by the type of mercury arc, voltage, and amperes employed, and distance of arc from slit no. I.

Heretofore, researches employing quantitative spectroscopy have been made, and in many instances the slit-widths employed have not been stated. Figure 4 contains the absorption curves for beta carotene with different slit-widths. At wave length 4520  $\text{\AA}$  the effect of opening the slits from 0.007 to 0.08 mm. was to increase the limits of the wave lengths emitted from the second slit from 1.7 to 19.5  $\text{\AA}$ . (This spectral region includes 75 per cent. of the wave lengths.) This decreased the value of alpha 10 per cent.

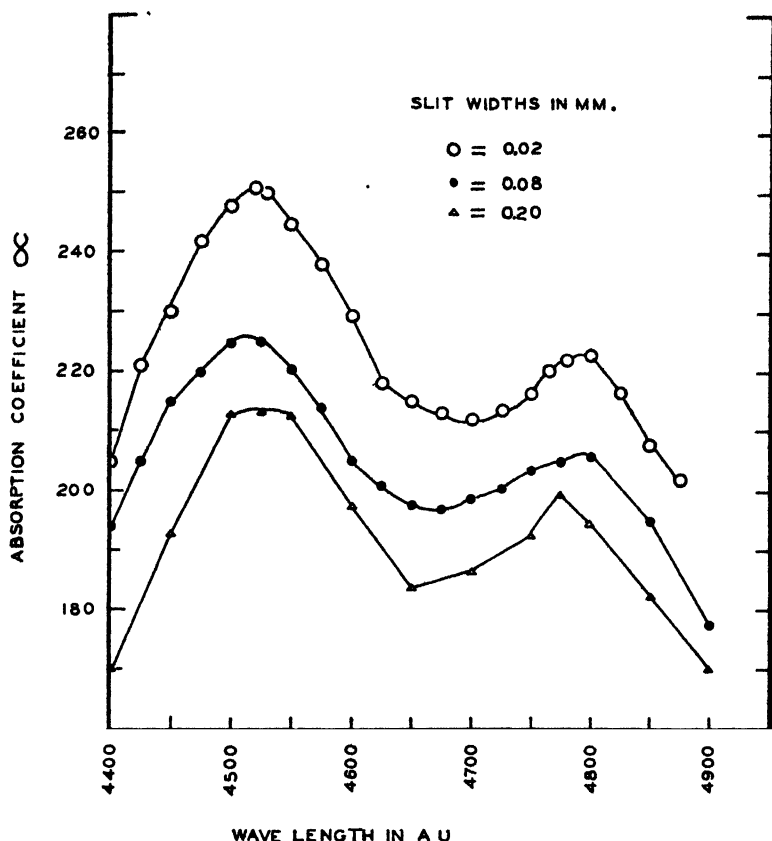


FIG. 4. Absorption spectra of  $\beta$  carotene at different slit-widths.

Figure 5 consists of curves representing the lowering of alpha, beta carotene, and lycopene by increasing the slit-widths. Similar curves were obtained when the mercury arc was employed as the light source. For the Zeiss monochromator, it would be useless to employ the standards presented in this paper with slit-widths wider than 0.05 mm. for quantitative analyses of the components within a group—even for estimating the total concentration for a group.

The value of alpha for compounds like benzene is affected more by slit-widths than those compounds with broad bands like the carotenoids and the inorganic standard presented in figure 3.

The simple method of determining whether "scattered" light (due to too wide slits) is of any importance is to replace the ordinary source of light by a monochromatic one, *i.e.*, mercury or sodium arc, without changing any of the other adjustments. This precaution should always be observed before quantitative measurements or analyses are made.

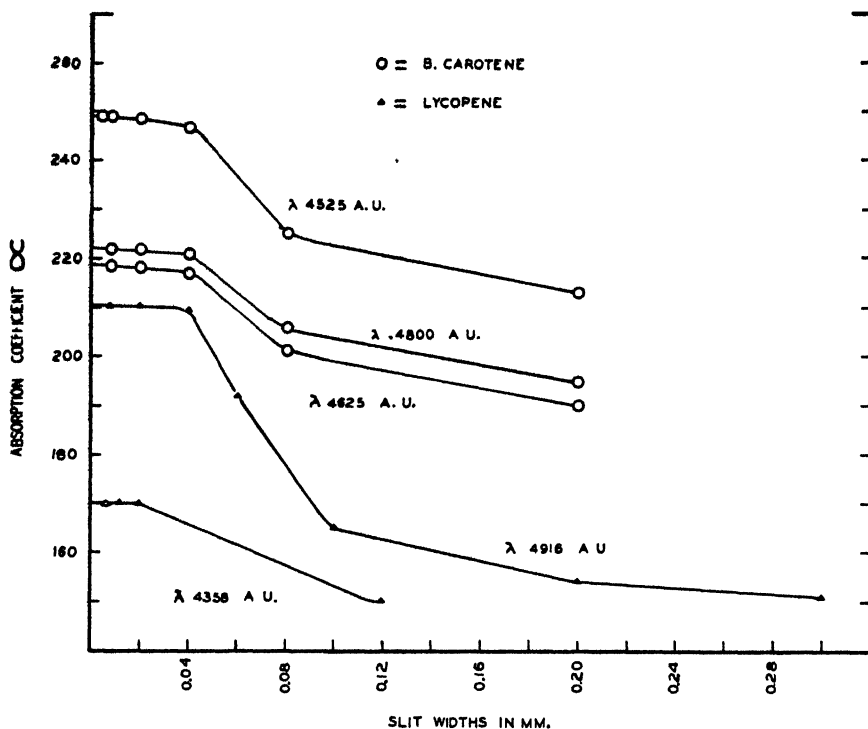


FIG. 5. The lowering of  $\alpha$  at different wave lengths with increasing slit-widths.

After the proper calibration has been made to check the linearity between the incident light on the photocell and the deflections of the galvanometer, another test for scattered light is to measure  $\alpha$  under conditions which give small and large values for  $I_0$  and  $I_x$  respectively—within concentration limits that obey Beer's law. If scattered light is a factor, the  $\alpha$ , in which  $I_x$  is small, will be low. That is, a constant error, such as due to scattered light, will be increased with a small  $I_x$ . Another reason for employing very narrow slit-widths is to reduce the rate of photochemical reactions to a minimum.

### Experimentation

#### PHOTOCHEMICAL DECOMPOSITION

In measuring absorption spectra of the common carotenoids, it is impossible in the ultraviolet region to eliminate photochemical reactions. In this study, the apparatus was so designed that by employing narrow slit-widths, it was hoped that the amount of photochemical decomposition could be decreased to less than the experimental error. At first, studies were made on the more stable carotenoids—alpha and beta carotene. In eight different

measurements of the absorption spectra no photochemical decomposition could be detected after irradiating the respective solutions 30 to 40 times in the course of determining the absorption spectra.

The next compound studied was lycopene. At the beginning of an experiment,  $\alpha$  was measured at wave length 3000 Å. After twenty subsequent measurements were made at 50-Å intervals throughout the entire ultraviolet region,  $\alpha$  was again measured at wave length 3000 Å. This value of  $\alpha$  checked with the first measurement. The error was 0.5 per cent. These experiments show that if very narrow slit-widths are employed, the amount of photochemical decomposition is insignificant, even for compounds as labile as lycopene.

The solvents employed in this investigation were purified as described by MILLER (6).

### ERRORS

The inherent errors of the circuit have already been discussed in connection with the description of the amplification circuit.

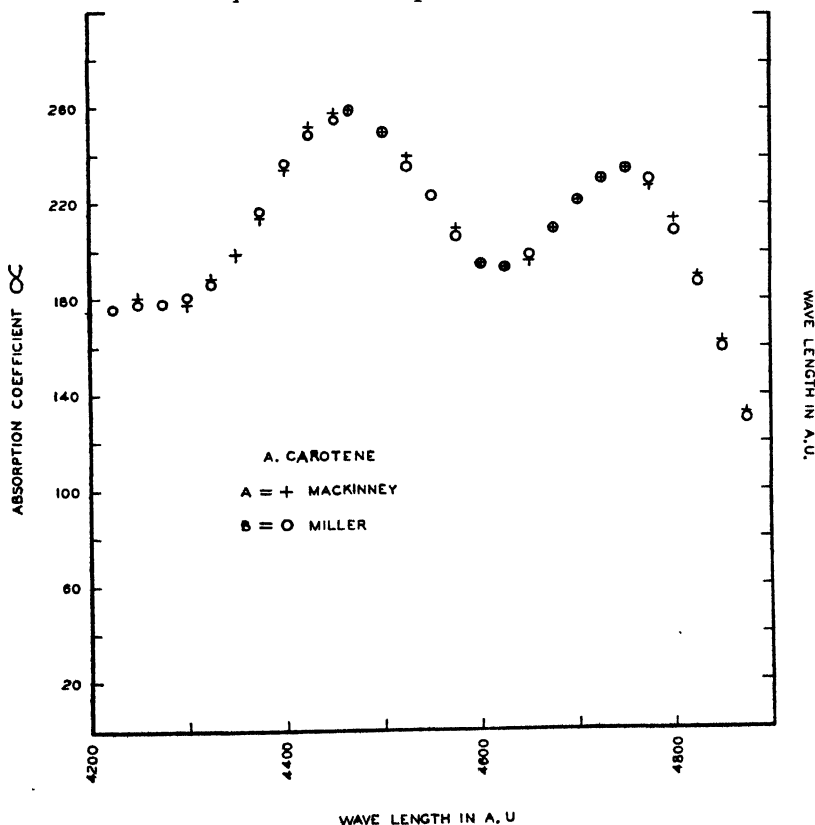


FIG. 6. Duplicate absorption curves measured by different manipulators.

A common criterion frequently employed in the purification of organic compounds is to regard the preparation with the largest absorption coefficient as being the purest. In the visible region, the discrepancy between purified preparations should be less than 1 per cent. In those regions where the absorption coefficient is less than 20 to 40, the difference between two samples may be 2 to 10 per cent. This increase in error is largely due to traces of impurities in the solvent and sample.

In the ultraviolet region the error is usually increased due to difficulties in keeping the absorption cells clean. Only in part can this factor be corrected. In this study the maximum error introduced by adsorption on the cell surfaces was 0.5 per cent. Likewise, the concentration range over which Beer's law is obeyed becomes more limited in the extreme ultraviolet. By employing different concentrations in the absorption cells, the concentration range over which Beer's law is obeyed is readily established.

Fortunately, the errors introduced by the apparatus, weighing, manipulators, balance, zero transmission of the absorption cells, dilutions, slit-width settings, and prism settings tend to cancel each other. In figure 6 this is illustrated by absorption curves A and B. In this experiment, two manipulators, working independently, measured the absorption of alpha carotene in the visible region. In view of the fact that the apparatus error was approximately  $\pm 0.4$  per cent. when this test was made, the agreement between the two sets of data is good. It is interesting to note that the largest discrepancies occurred at wave lengths 4350 and 4800 Å. At these wave lengths the slope of the absorption curve is large, and consequently a slight error in the prism settings would introduce a significant error.

#### ABSORPTION SPECTRA

The absorption spectra of alpha carotene, beta carotene, and lycopene have been quantitatively measured between wave lengths 2200 and 5400 Å. The absorption curves are presented in figure 7.<sup>6</sup> In this study, the precautions described by Miller (6) have been employed.

In the carotenoid literature the largest discrepancies occur between absorption spectra data obtained by investigators in different laboratories. The absorption coefficients of the carotenes and lycopene were first measured by KUHN (3) and SMAKULA (8). When these data were published, no calibration data were presented. MILLER (6) and SMITH (9) were unable to confirm the values obtained by KUHN and SMAKULA, although SMITH's (9) curves agreed with those curves previously reported by MILLER within the experimental error. SMITH (9), in a detailed study concerning sources of errors, was unable to satisfactorily explain the discrepancies in absorption spectra data reported by KUHN and SMAKULA, and MILLER and SMITH.

<sup>6</sup> In figure 7, the experimental values are presented, usually at 50-Å intervals because the graph was greatly reduced for publication.

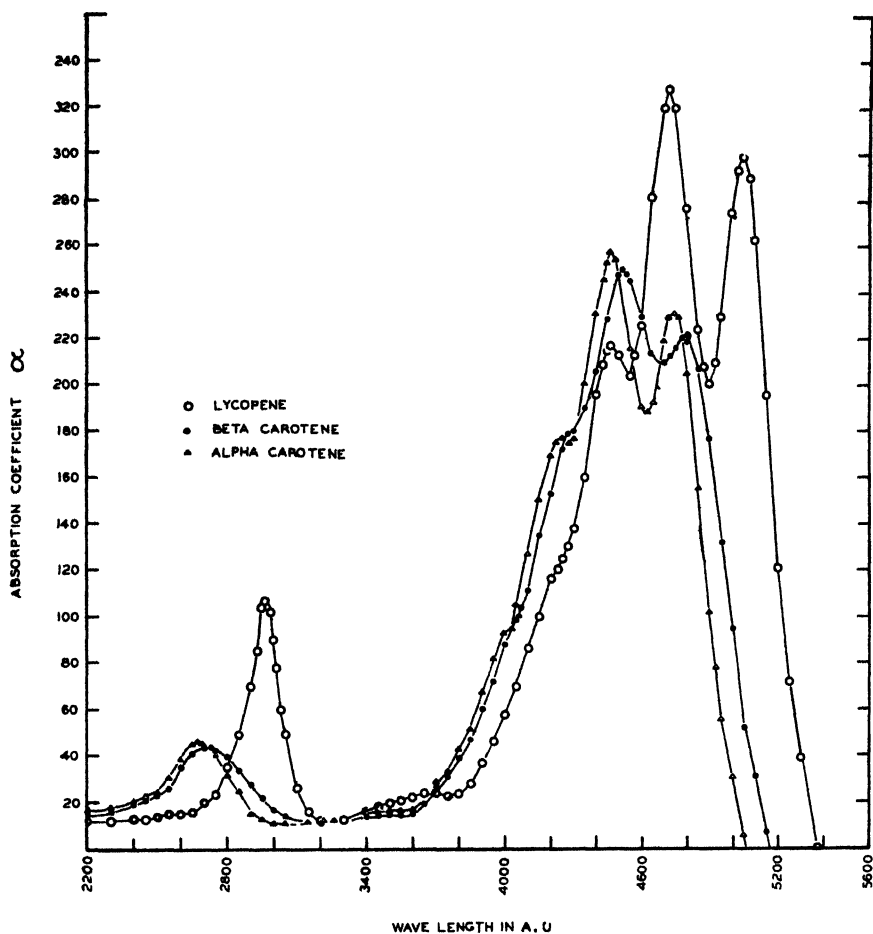


FIG. 7. Absorption spectra of  $\alpha$  carotene,  $\beta$  carotene, and lycopene in 80 per cent. ethanol and 20 per cent. diethyl ether.

Readings were taken at 25-Å intervals, save 10-Å intervals over the maxima and minima.<sup>6</sup> The absorption spectrum of alpha carotene consists of four bands and a shelf. The maxima for the bands occurs at 4750 Å ( $\alpha = 231.5 \pm 1.5$ ), 4465 Å ( $\alpha = 258 \pm 1.5$ ), 4246 Å ( $\alpha = 178 \pm 1.6$ ), and 2675 Å ( $\alpha = 47.0 \pm 0.5$ ). The shelf occurs at 4010 Å. In the visible region a minimum occurs at 4625 Å ( $\alpha = 190.0 \pm 1$ ). The extremely wide minimum in the ultraviolet (approximately the same for the carotenoids investigated in this study) will be discussed under oxidation.

The absorption curve for beta carotene has three definite maxima at wave lengths 4800 Å ( $\alpha = 222 \pm 1.0$ ), 4525 Å ( $\alpha = 249 \pm 1.4$ ), and 2790 Å ( $\alpha = 43.0 \pm 0.5$ ). An apparent point of inflection occurs at 4055 Å and a

distinct shelf at 4300 Å ( $\alpha = 180.0 \pm 1.0$ ). A minimum is observed at 4700 Å ( $\alpha = 210.0 \pm 1.0$ ).

The lycopene was isolated from tomatoes, and furnished by Dr. M. B. MATLACK (4). Lycopene has four bands with well-defined maxima at wave lengths 5050 Å ( $\alpha = 298 \pm 2$ ), 4725 Å ( $\alpha = 328 \pm 2$ ), 4465 Å ( $\alpha = 217 \pm 1$ ), and 2960 Å ( $\alpha = 102.5 \pm 2$ ). In the visible spectrum, minima were found at 4900 Å ( $\alpha = 201.5 \pm 2$ ) and at 4550 Å ( $\alpha = 204 \pm 2$ ). A small shelf was noted between wave lengths 4200 and 4250 Å.

#### OXIDATION OF CAROTENOID SOLUTIONS

A detailed spectroscopic study has been made by McNICHOLAS (5) on the oxidation of carotene (chiefly beta) and "leaf xanthophyll" in 80 per cent. ethanol and 20 per cent. ether. McNICHOLAS reports that during the first "stages" of oxidation, there is a marked increase in a band at wave length 3410 Å. He concludes that his purest carotene samples exhibited a slight inflection at this same wave length.

In figure 7 the carotene curves also show a very small inflection between wave lengths 3400 and 3500 Å, and a similar inflection for lycopene at wave length 3700 Å. For the carotenes, this inflection is no greater than the experimental error; hence insignificant, save that many of the earlier studies report a distinct band. Therefore a few experiments were made to observe the rate at which these bands develop.

After the absorption coefficients of beta carotene and lycopene had been measured (fig. 7) the rate of oxidation was followed spectroscopically by the development of bands between wave lengths 3400 and 3800 Å. When the values of the alphas at the respective maxima had increased twofold, the absorption curve for beta carotene and lycopene were measured. These data are presented in figure 8. Curve B shows that on the fifth day after the carotene was dissolved in the solvent, the value of the absorption coefficients at wave length 3400 Å had increased twofold. On the second day, the value of alpha (curve E) for lycopene had increased from 20 to 43 at wave length 3650 Å.

Additional experiments were made to determine the rate of oxidation of the lycopene solution when the absorption cells were placed in front of slit no. 1, so that the lycopene solution was exposed to the total irradiation of the discharge tube. When the lycopene solution had been exposed less than 20 minutes, the value of alpha at wave length 3600 Å was larger than the absorption coefficient at wave length 2960 Å.

#### SIGNIFICANCE OF OXIDATION EXPERIMENTS

**CAROTENES.**—KUHN (3) and SMAKULA (8) in their spectroscopic investigations of the carotenes failed to observe an inflection in the absorption

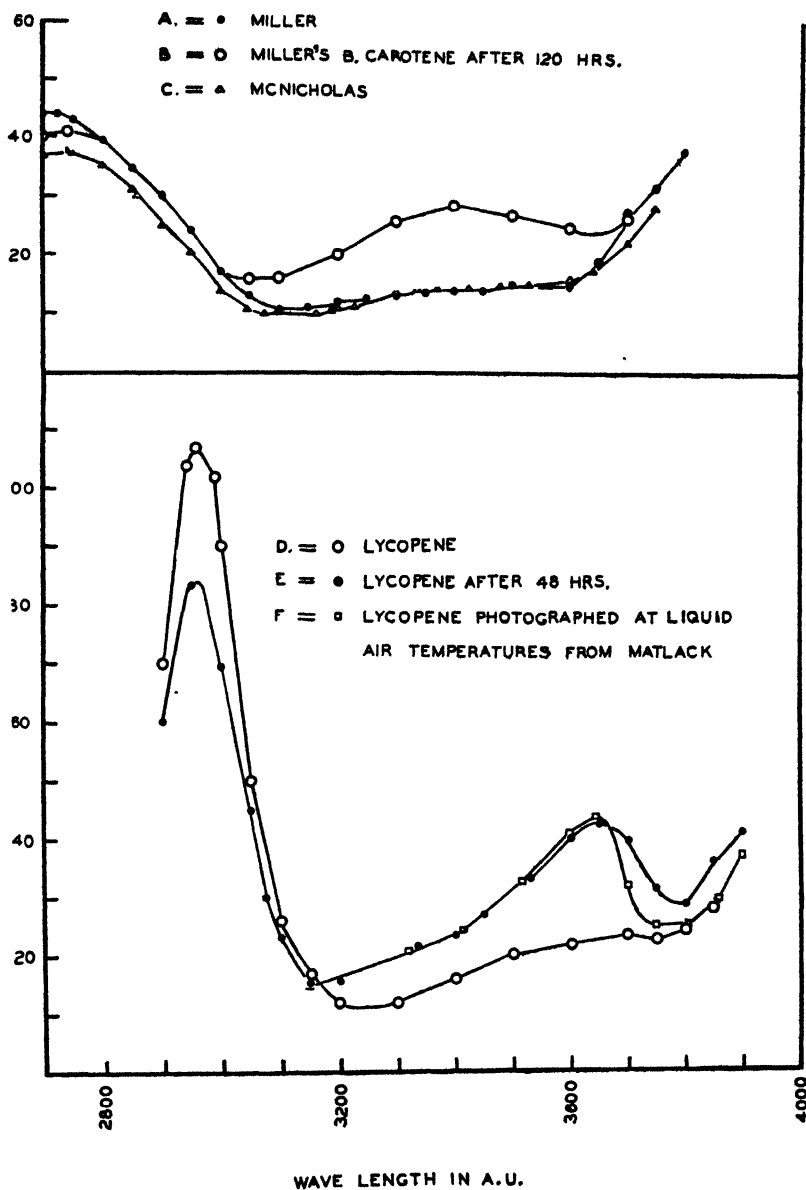


Fig. 8. Changes in  $\beta$  carotene and lycopene spectra accompanying oxidation.

curve of either alpha or beta carotene in hexane. KUHN's carbon and hydrogen analyses demonstrated that the respective carotene samples were pure. He also employed a hydrocarbon as solvent, which would cause a slower rate of oxidation than a mixture of ethanol and ether.



Since KUHN employed aluminum oxide (Fasertonerde) in his method of purifying the carotenes, the 3400-Å inflection (band) is not due to oxidative changes introduced by the adsorbent, but rather by the solvent, oxygen, and light.

The small inflection observed by McNICHOLAS (5) no doubt is due to impurities, because the value of  $\alpha$  at wave length 4250 Å for his carotene sample is 1.5 to 2 per cent. lower than that presented in figure 7. This discrepancy is of the same magnitude as that presented by his increase at wave length 3400 Å.

LYCOPENE.—For some time it has been known that lycopene is readily oxidized, even more so than the carotenes. This is demonstrated in a study by SMAKULA (8). He measured the absorption curve in hexane and, although he found a distinct band at wave length 3650 Å, he does not record it as such. MATLACK (4) succeeded in isolating pure lycopene from tomatoes—purity based on carbon and hydrogen analyses. His associates measured the absorption coefficients of lycopene in visible and ultraviolet region at liquid air temperatures. MATLACK obtained evidence that a lycopene dissolved in ethanol and ether was partially oxidized after standing 12 hours in the dark. The data in figure 7 confirm the absorption curve reported by MATLACK for the visible region. In the ultraviolet region MATLACK reports bands at wave lengths 3650 and 2960 Å.

After the lycopene curve in figure 7 was measured, the absorption coefficients were again determined on 48-hour-old lycopene solutions. These data are presented in figure 8, curve E. The absorption curve reported by MATLACK and SANDO was measured photographically and is, in part, represented by curve F.<sup>7</sup> It is interesting to note that even at liquid air temperatures and a time interval as short as that necessary for a spectrogram, approximately as much photooxidation occurred as that (oxidation) occurring when a similar solution is allowed to stand 2 days at 20° C. These data suggest that the band (or inflection) at wave length 3650 Å, as reported by SMAKULA, MATLACK, and SANDO, is due to oxidation. Likewise the small inflection in the lycopene curve in figure 7 is due to slight traces of oxidation—inflection approximately that of the experimental error.<sup>8</sup>

### Summary

1. A precise method has been developed for the quantitative measurement of absorption coefficients. Data are presented to show that for carotenoid research the absorption cells must be placed behind slit no. 2. When

<sup>7</sup> The curve is reproduced as accurately as it is possible to read the values from figure 4 in reference (4).

<sup>8</sup> While this paper was in press, a paper by HOGNESS, ZSCHEILE, and SIDWELL has been published in the March, 1937 issue of *Physical Chemistry*. This paper concerns the construction, calibration, and the use of a photoelectric spectrophotometer in chemistry.

this precaution is observed and narrow spectral regions are employed, the photooxidation is so decreased that, during the measurement of an absorption curve, the total decomposition is less than the experimental error.

2. A procedure for a detailed calibration of the photoelectric apparatus has been discussed. Suggestions are made which will make it easier for investigators in different laboratories to obtain concordant results.

3. Calibrated filters obtained from the Bureau of Standards are the best single test to ascertain that the apparatus will give correct measurements.

4. Precautions and limitations are discussed as to when it is not possible to employ the standards, presented in this paper, for quantitative analysis.

5. The sources of errors are discussed. When the same apparatus is employed by several investigators, many of the possible errors cancel each other. The magnitude of the total errors in the various parts of the visible and ultraviolet region has been stated.

6. The absorption coefficients in the visible and ultraviolet region have been measured in 80 per cent. ethanol and 20 per cent. ether for alpha carotene, beta carotene, and lycopene.

7. Data have been presented showing that a band at wave length 3400 Å, or even an inflection, is due to traces of carotene oxidation products present.

8. Photo- or chemical-oxidation or both are responsible for the absorption band at wave length 3650 Å, as previously reported for lycopene.

9. In the ultraviolet region, even at liquid air temperatures, it is impossible to obtain an accurate spectrogram of lycopene.

10. The bearing of the data presented in this paper, to that reported by other investigators has been discussed.

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# ENZYME ACTIVITY IN COLD-HARDENED AND UNHARDENED RED CLOVER<sup>1</sup>

GLENN A. GREATHOUSE AND NEIL W. STUART

## Introduction

The ultimate objective of winter-hardiness investigations is to find the fundamental nature of this phenomenon. Toward that end results are reported of a study on the autolysis of red clover root and crown tissue when in the cold-hardened and unhardened condition. Results are also reported from experiments on hardened red clover plants stored for 11 days at temperatures of 34°, 31° and 12° F. Carbohydrate and nitrogen analyses were made on these tissues. It has been suggested (8, 9) that the sugars are related to protein precipitation, thus a study of the activity of the proteolytic and carbohydrate enzymes under similar conditions would appear to be of great interest and value.

## Literature review

An extended review of the literature on various enzymes is outside the scope of this paper. The most recent work on enzymes as related to winter-hardiness is that of TYSDAL (11) on diastatic activity in alfalfa plants. NEWTON and BROWN (7) and others have noted a relation of winter-hardiness and catalase activity, the hardier variety having the higher activity.

The importance of sugars in the hardening process has been suggested by LIDFORSS (5), AKERMAN (1), NEWTON and BROWN (8), SCHAFFNIT (9) and others. Higher concentrations of sugars are associated with winter-hardiness in clovers (2, 3) and the question immediately arises, what changes occur within the plant to promote and retain the increase of sugars? TYSDAL (11) studied the diastatic power of the alfalfa plant as related to the hardened-off condition. In his investigation only the diastatic enzymes were studied. This still leaves the question unanswered as to whether the increase in sugars during the hardening period is due to conversion of starch to sugar, or whether the increase in sugar is not at the expense of starch, as TUMANOW (10) and others have suggested. Or, again, it may be that the hardier variety is more conservative of its already formed sugar, possibly because of a lower respiration rate.

KLING (4) reported that winter cereals could be differentiated as to cold hardiness on the basis of their protein ferments.

<sup>1</sup> Contribution from the University of Maryland, College Park, Maryland.

### Materials and methods

The Ohio and French varieties of red clover<sup>2</sup> were selected as types that are winter hardy and less hardy respectively. The plant material for the autolysis experiment was grown on experimental plots which were seeded on April 21, 1934, on the Maryland Experiment Station Farm at College Park, Maryland. A 2-12-4 fertilizer was applied at the rate of 600 lb. per acre to the sandy loam soil previous to seeding. The seed was inoculated and planted without a companion crop at the rate of 15 lb. per acre. The plants were clipped at a height of 4 in. on September 12, 1934.

On January 30, 1935, both varieties of clover plants were removed from the snow-covered ground and separated into roots and crowns. All of the living shoot tissue was included with the crowns. The tissue was prepared for sampling as previously described (2). Suitable portions of the macerated tissue were placed in Kohlrausch or Erlenmeyer flasks, thoroughly wetted with toluene-saturated water, plugged with cotton, and placed in an oven at  $37 \pm 0.2^\circ \text{C}$ . One set of the samples was analyzed immediately and others after autolysis periods of 24, 48 or 120 hours. An additional set of samples was placed in a water bath at  $70^\circ \text{C}$ . for 1 hour and then transferred to the  $37^\circ \text{C}$ . oven for 24 hours. Another set of samples was frozen at  $-22^\circ \text{C}$ . for 1 hour and then placed at  $37^\circ \text{C}$ . for 24 hours. Care was taken that the samples were saturated with toluene water at all times during the autolysis at  $37^\circ \text{C}$ . The experiment was repeated on March 25, 1935, the only change being that some of the autolysis periods were shortened. At this time the plants were in the early spring stage just starting to grow.

The same varieties of clovers were seeded in rows in cypress boxes on October 14, 1935, and grew in the greenhouse until January 29, 1936. On this date the boxes containing the clover plants were placed in a cold storage room<sup>3</sup> which maintained a temperature of  $34 \pm 2^\circ \text{F}$ . These plants received continuous light from four 500-watt Mazda lamps, placed at a distance of 3 feet from the plants. On February 11, 1936, the plants were divided into four uniform lots of 4 boxes each. Lot 1 remained at  $34^\circ \text{F}$ . and lots 2 and 3<sup>4</sup> were placed at  $31 \pm 1^\circ \text{F}$ ., and  $12 \pm 1^\circ \text{F}$ ., respectively. Lot 4 was taken for analysis on February 11 and designated as check in the tables. Lots 1, 2, and 3 did not receive illumination during the 11 days of storage as all storage rooms were dark.

### Experimental results

The results of the carbohydrate analyses are shown in tables I to VI.

<sup>2</sup> Ohio F.C.I. Accession no. 20195; French, Accession no. 20141. The letters F.C.I. indicate the accession number of the Division of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

<sup>3</sup> Through the courtesy of the Department of Horticulture, University of Maryland.

<sup>4</sup> Cold storage room, Arlington Farm, Va., through the courtesy of the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry.

In the January autolysis experiment the invertase activity was greater in the Ohio roots than in the French roots. The data show that the starch and dextrin content of the Ohio roots decreased 30.2 per cent., while the French roots decreased 41.5 per cent. during the first 24 hours of autolysis, as measured by the percentage decrease over the original or check analysis (table III). The Ohio and French crown tissue decreased 63.5 per cent. and 94.9 per cent. respectively, in the starch and dextrin fraction over the same period of time. Heating the tissue in January to 70° C. for 1 hour did not destroy the carbohydrate enzymes, although accumulation of reducing sugars was not quite so great as in the untreated samples. The enzymatic activity for the starch and dextrin fraction was stimulated in all samples except French crowns by heating to 70° C. for 1 hour previous to the 24 hours of autolysis. The data indicate that the carbohydrate enzymes in the French tissue were injured more than those in the Ohio tissue by the heat treatment.

TABLE I

DISTRIBUTION OF CARBOHYDRATES IN RED CLOVER ROOTS AND CROWNS SAMPLED JANUARY 30, 1935. RESULTS ARE EXPRESSED AS PERCENTAGE OF THE DRY WEIGHT

PERIOD OF AUTOLYSIS	REDUCING SUGARS	SUCROSE	TOTAL SUGARS	STARCH AND DEXTRINS	HEMICEL- LULOSE
<i>hours</i>	%	%	%	%	%
OHIO ROOTS					
(Check) 0 .....	2.06	10.65	13.40	13.55	8.89
24 .....	19.13	0.0	19.34	9.32	6.78
1 hr. 70° C.; 24 hr. 37° C. ....	18.86	0.69	20.25	4.82	7.93
1 hr. -22° C.; 24 hr. 37° C. ....	18.84	1.27	20.84	9.81	7.31
48 .....	21.05	0.23	22.12	9.66	6.27
FRENCH ROOTS					
(Check) 0 .....	1.92	8.40	10.86	8.81	9.86
24 .....	16.07	0.24	16.93	5.16	8.94
1 hr. 70° C.; 24 hr. 37° C. ....	15.02	0.65	16.25	4.35	8.94
1 hr. -22° C.; 24 hr. 37° C. ....	15.49	0.39	16.48	5.07	8.66
48 .....	18.67	0.96	20.60	5.42	7.86
OHIO CROWNS					
(Check) 0 .....	4.02	6.33	10.88	4.47	9.36
24 .....	14.53	0.18	15.30	1.63	8.21
1 hr. 70° C.; 24 hr. 37° C. ....	12.55	0.02	13.05	1.37	9.24
1 hr. -22° C.; 24 hr. 37° C. ....	14.65	0.0	14.53	1.80	7.73
48 .....	15.42	0.63	16.79	1.57	6.72
FRENCH CROWNS					
(Check) 0 .....	3.20	5.15	8.78	2.30	10.25
24 .....	11.70	0.42	12.41	0.12	9.64
1 hr. 70° C.; 24 hr. 37° C. ....	9.54	0.12	10.11	0.92	10.51
1 hr. 22° C.; 24 hr. 37° C. ....	12.07	0.28	12.87	0.15	9.05
48 .....	12.38	0.18	13.22	0.11	7.98

Freezing the tissue at  $-22^{\circ}\text{C}$ . for 1 hour previous to autolysis had little, if any, effect upon the carbohydrate enzymatic activity (tables I, II).

TABLE II

DISTRIBUTION OF CARBOHYDRATES IN RED CLOVER ROOTS AND CROWNS SAMPLED MARCH 25, 1935. RESULTS ARE EXPRESSED AS PERCENTAGE OF DRY WEIGHT

PERIOD OF AUTOLYSIS	REDUCING SUGARS	SUCROSE	TOTAL SUGARS	STARCH AND DEXTRINS	HEMICEL- LULOSE
<i>hours</i>	%	%	%	%	%
OHIO ROOTS					
(Check) 0	2.33	2.54	5.22	3.95	11.82
6	7.05	0.0	7.34	2.12	9.78
24	9.37	0.34	10.10	1.65	8.61
1 hr. $70^{\circ}\text{C}$ .; 24 hr. $37^{\circ}\text{C}$ .	8.09	0.0	8.43	1.49	10.47
FRENCH ROOTS					
(Check) 0	1.74	0.59	2.52	1.88	12.23
6	4.23	0.0	4.28	0.79	10.21
24	5.23	0.40	6.05	0.76	8.87
1 hr. $70^{\circ}\text{C}$ .; 24 hr. $37^{\circ}\text{C}$ .	3.41	0.0	3.59	1.01	11.08
OHIO CROWNS					
(Check) 0	3.35	0.13	3.20	2.33	11.41
6	4.92	0.0	5.03	1.02	7.64
24	6.66	0.13	7.08	0.92	7.46
1 hr. $70^{\circ}\text{C}$ .; 24 hr. $37^{\circ}\text{C}$ .	4.02	0.0	4.25	1.07	10.17
FRENCH CROWNS					
(Check) 0	2.23	0.16	2.56	2.06	9.89
6	3.69	0.0	3.91	0.84	7.66
24	5.76	0.06	6.11	0.77	6.58
1 hr. $70^{\circ}\text{C}$ .; 24 hr. $37^{\circ}\text{C}$ .	2.31	0.0	2.49	1.68	9.24

In March, after growth had started, the clover roots and crowns were lower in total sugars, starch and dextrins than in January. The autolysis periods for March were 6 and 24 hours, respectively, in contrast to 24 and 48 hours for January. After 24 hours of autolysis on the tissues sampled in March the differences between the varieties tended to disappear. As in January, greater increases in reducing sugars were observed in the Ohio roots than in the French roots upon autolysis. Conversely the starch and dextrin fraction decreased more in the French roots than in the Ohio roots. Similarly to the January sample the reducing sugar increase and the starch and dextrin decrease were proportionally greater in the French crowns, although the differences were not as great for the March analysis. Following 6 hours of autolysis the starch and dextrin fraction had decreased 46.3 per cent. in the Ohio roots and 58.1 per cent. in the French roots. Decrease in the starch

and dextrin fraction of the Ohio and French crowns of 56.5 per cent. and 59.2 per cent., respectively, was noted over a similar period of autolysis. Heating the tissue sampled in March to 70° C. for 1 hour previous to autolysis decreased the enzymatic activity in all cases, except for the starch and dextrin hydrolysis in the Ohio root tissue. There was apparently less protection of the enzymes in March than in January. The protective action was very poor in the French crown tissue in both January and March. Data from tables I, II, VII, and IX show that there was about twice as much total sugar, amino acids, etc., in both varieties in January as in March.

TABLE III

DISTRIBUTION OF CARBOHYDRATES IN RED CLOVER ROOTS AND CROWNS SAMPLED JANUARY 30, 1935. RESULTS ARE EXPRESSED AS PERCENTAGE OF THE ORIGINAL, TAKEN AS 100 PER CENT.

PERIOD OF AUTOLYSIS	REDUCING SUGARS	SUCROSE	TOTAL SUGARS	STARCH AND DEXTRINS	HEMICEL- LULOSE
<i>hours</i>	%	%	%	%	%
OHIO ROOTS					
24 .....	927.0	0.0	144.3	69.8	76.3
1 hr. 70° C.; 24 hr. 37° C. ....	914.0	6.5	151.5	36.1	89.2
1 hr. -22° C.; 24 hr. 37° C. ....	913.2	11.9	155.5	73.4	82.2
48 .....	1020.5	2.2	165.1	72.3	70.5
FRENCH ROOTS					
24 .....	837.3	2.9	155.9	58.5	90.7
1 hr. -70° C.; 24 hr. 37° C. ....	782.4	7.7	149.7	49.4	90.6
1 hr. -22° C.; 24 hr. 37° C. ....	807.3	4.7	151.8	57.5	87.8
48 .....	973.0	11.4	189.7	61.5	79.6
OHIO CROWNS					
24 .....	361.3	2.7	140.6	36.5	87.6
1 hr. 70° C.; 24 hr. 37° C. ....	312.1	0.3	119.9	30.7	98.7
1 hr. -22° C.; 24 hr. 37° C. ....	364.4	0.0	133.5	40.2	82.6
48 .....	383.8	9.9	154.2	35.1	71.7
FRENCH CROWNS					
24 .....	365.8	8.1	141.3	5.1	93.9
1 hr. 70° C.; 24 hr. 37° C. ....	298.4	2.3	115.1	40.1	101.0
1 hr. -22° C.; 24 hr. 37° C. ....	337.4	5.5	146.6	6.4	88.3
48 .....	387.2	3.6	150.5	4.6	77.4

A comparison of relative enzymatic activity in January and in March can be noted in tables III and IV. It is not possible to compare the increases in reducing sugars at the two periods, since the amount of total sugars, starch, etc., was not the same at these dates. However, the percentage of decrease in the starch and dextrin fraction is of interest and indicates the enzymatic



TABLE IV

DISTRIBUTION OF CARBOHYDRATES IN RED CLOVER ROOTS AND CROWNS SAMPLED MARCH 25, 1935. RESULTS ARE EXPRESSED AS PERCENTAGE OF THE ORIGINAL, TAKEN AS 100 PER CENT.

PERIOD OF AUTOLYSIS	REDUCING SUGARS	SUCROSE	TOTAL SUGARS	STARCH AND DEXTRINS	HEMICELLULOSE
<i>hours</i>	%	%	%	%	%
OHIO ROOTS					
6 .....	302.5	0.0	140.7	53.7	82.8
24 .....	401.8	1.3	193.5	41.7	72.8
1 hr. 70° C.; 24 hr. 37° C. ....	347.1	0.0	161.7	37.7	88.5
FRENCH ROOTS					
6 .....	242.4	0.0	169.9	41.9	83.4
24 .....	299.8	0.7	240.1	40.4	72.5
1 hr. 70° C.; 24 hr. 37° C. ....	195.5	0.0	142.5	53.7	90.5
OHIO CROWNS					
6 .....	146.6	0.0	157.1	43.5	66.9
24 .....	198.5	1.1	220.9	39.3	65.4
1 hr. 70° C.; 24 hr. 37° C. ....	119.9	0.0	132.7	46.0	89.1
FRENCH CROWNS					
6 .....	165.7	0.0	152.7	40.8	77.4
24 .....	258.9	0.4	238.7	37.2	66.5
1 hr. 70° C.; 24 hr. 37° C. ....	103.5	0.0	99.1	81.3	93.3

activity on this fraction at the two periods. The percentage of decrease in starch and dextrin in the Ohio root for January was 31.2 per cent. and in March 46.3 per cent. Similarly in the French root the starch and dextrin fraction decreased 41.5 per cent. in January and 58.1 per cent. in March. An autolysis period of 24 hours in March produced hydrolysis of about 60 per cent. of the starch and dextrin in both Ohio and French roots.

In January 63.5 per cent. of the starch and dextrins in the Ohio crowns were hydrolyzed in 24 hours. For the same period 94.9 per cent. of starch and dextrins in the French crowns were hydrolyzed. An autolysis period of 24 hours in March brought about hydrolysis of nearly 62 per cent. of the starch and dextrins in both Ohio and French crowns.

The distribution of the carbohydrates in red clover roots stored for 11 days in darkness at low temperatures is shown in table V. These plants were undisturbed until sampled for analysis. At all storage temperatures the Ohio roots maintained a larger percentage of total sugars, starch, and dextrins than did the French roots. The reverse relationship exists for the pentosan and hemicellulose fractions of these tissues. The apparent per-

TABLE V

DISTRIBUTION OF CARBOHYDRATES IN RED CLOVER ROOTS. RESULTS ARE EXPRESSED AS PERCENTAGE OF THE DRY WEIGHT

SAMPLING DATE	VARIETY	STORAGE TEMPERATURE	REDUCING SUGARS	SUCROSE	TOTAL SUGARS	STARCH AND DEXTRINS	PENTOSANS	HEMICEL- LULOSE
		°F.	%	%	%	%	%	%
1936								
Feb. 11	Ohio	34 (check)	2.40	3.13	5.84	1.78	7.61	8.50
	French	34 (check)	3.33	4.11	7.85	1.63	7.12	8.47
Feb. 22	Ohio	34	1.71	7.32	9.56	1.03	7.03	8.13
" "	French	34	1.81	4.65	6.87	0.50	8.60	8.75
" "	Ohio	31	2.41	8.39	11.40	1.43	7.36	8.40
" "	French	31	2.23	6.93	9.68	1.21	7.64	8.91
" "	Ohio	12	3.31	1.40	4.94	1.04	8.21	8.60
" "	French	12	3.30	1.11	4.64	0.80	9.68	9.79

centage of increase and decrease of the pentosans and hemicellulose follow the decrease and increase of the total solids. This experiment was of considerable value and interest in that it showed that the changes that occurred in the roots of the undisturbed clover plants, when placed in darkness at temperatures of 34°, 31°, and 12° F., were similar to those in macerated tissue (autolysis). Especially is this similarity noted in the starch and dextrins fraction. The reducing sugars of both varieties of clover increased with the lowering of the temperature, the Ohio variety always maintaining the lead. The non-reducing sugars increased with the lowering of the temperature from 34° to 31° F., and then decreased from 31° to 12° F. The rate of starch and dextrin hydrolysis was greater in both varieties of clover at 34° and 12° F. than at 31° F.

The results of the nitrogen analyses are shown in table VII to XII. During autolysis a portion of the total protein in the roots and crowns is hydrolyzed into amino acids, bases, etc. The portion of the protein removed during the extraction of the non-protein (designated extracted protein) decreased in both root and crown tissue during the entire period of autolysis. The greatest decrease was during the first 24 hours of autolysis. Conversely the non-protein nitrogen increased. This increase in amino nitrogen at successive autolysis periods was fairly regular. However, basic nitrogen accumulated only during the later autolysis periods. There was little increase in the amide and ammonia fraction at any time.

In the January autolysis experiment the increase in non-protein nitrogen in both the Ohio and French roots was somewhat greater than the decrease in extracted protein. However, proteolytic enzyme activity was greater in the Ohio roots than in the French roots, as measured by the percentage increases in non-protein nitrogen over the original or check analysis (table VIII). Heating the sample to 70° C. for 1 hour did not destroy the proteolytic enzymes, although accumulation of non-protein nitrogen was not so great as in the untreated samples. The enzymes in the French roots were injured more than those in the Ohio roots by the heat treatment.

In the clover crown tissue which contained more total and protein nitrogen than the roots, there was a greater decrease in the extracted protein than an increase in the non-protein nitrogen. In further contrast with the clover roots, proteolytic enzyme activity was greater in the French crowns than in the Ohio crowns.

In March, after growth had started, the clover roots and crowns were lower in total nitrogen than in January. Proteolytic activity came to an equilibrium after 48 hours of autolysis in roots of both varieties. As in January, greater increases in non-protein nitrogen were observed in the Ohio roots than in the French roots. This increase was much greater than the decrease in extracted protein. Evidently the reserve proteins more intimately associated with the cellular structure were undergoing proteolysis.

TABLE VI

DISTRIBUTION OF CARBOHYDRATES IN RED CLOVER ROOTS. RESULTS ARE EXPRESSED AS PERCENTAGE OF THE ORIGINAL, TAKEN AS 100 PER CENT.

SAMPLING DATE	VARIETY	STORAGE TEMPERATURE	REDUCING SUGARS	SUCROSE	TOTAL SUGARS	STARCH AND DEXTRINS	PENTOSANS	HEMICELLULOSE
1936		°F.	%	%	%	%	%	%
Feb. 22	Ohio	34	71.3	233.9	163.7	57.9	92.4	95.6
" "	French	34	54.4	113.1	87.5	30.7	120.8	103.3
" "	Ohio	31	100.4	268.1	195.2	80.3	96.7	98.8
" "	French	31	66.7	168.6	123.3	74.2	107.3	105.2
" "	Ohio	12	137.9	44.7	84.6	58.4	107.9	101.2
" "	French	12	99.1	27.0	59.1	49.1	136.0	115.6

TABLE VII

DISTRIBUTION OF NITROGEN IN RED CLOVER ROOTS AND CROWNS SAMPLED JANUARY 30, 1935. RESULTS ARE EXPRESSED AS PERCENTAGE OF THE FRESH WEIGHT

PERIOD OF AUTOLYSIS Hours	TOTAL		PROTEIN		EXTRACTED		NON-PROTEIN		ALPHA-AMINO		BASIC		AMIDE		RESIDUAL	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
OHIO ROOTS																
(Check) 0	0.630		0.337		0.147		0.293		0.125		0.079		0.056		0.112	
24	.....		0.247		0.089		0.383		0.157		0.071		0.064		0.162	
1 hr. 70° C.; 24 hr. 37° C.	.....		0.261		0.086		0.369		0.141		0.076		0.066		0.162	
48	.....		0.251		0.061		0.379		0.165		0.099		0.066		0.148	
120	.....		0.178		0.049		0.452		0.199		0.186		0.076		0.177	
FRENCH ROOTS																
(Check) 0	0.544		0.296		0.129		0.248		0.108		0.065		0.052		0.088	
24	.....		0.236		0.068		0.308		0.134		0.063		0.056		0.118	
1 hr. 70° C.; 24 hr. 37° C.	.....		0.274		0.059		0.270		0.112		0.052		0.053		0.105	
48	.....		0.216		0.064		0.328		0.147		0.067		0.062		0.119	
120	.....		0.207		0.045		0.337		0.152		0.107		0.062		0.123	
OHIO CROWNS																
(Check) 0	0.649		0.402		0.218		0.247		0.118		0.060		0.066		0.063	
24	.....		0.363		0.079		0.286		0.135		0.049		0.066		0.085	
48	.....		0.332		0.062		0.317		0.153		0.075		0.070		0.094	
FRENCH CROWNS																
(Check) 0	0.593		0.384		0.206		0.209		0.100		0.056		0.055		0.054	
24	.....		0.323		0.098		0.270		0.119		0.050		0.058		0.093	
48	.....		0.305		0.079		0.288		0.133		0.063		0.063		0.092	

TABLE VIII

DISTRIBUTION OF NITROGEN IN RED CLOVER ROOTS AND CROWNS SAMPLED JANUARY 30, 1935. RESULTS ARE EXPRESSED AS PERCENTAGE OF THE ORIGINAL, TAKEN AS 100 PER CENT.

PERIOD OF AUTOLYSIS	PROTEIN N	EXTRACTED PROTEIN N	NON- PROTEIN N	ALPHA- AMINO N	BASIC N	AMIDE N	RESIDUAL N
	%	%	%	%	%	%	%
OHIO ROOTS							
24 .....	73.3	60.5	130.7	125.6	89.9	114.3	144.6
1 hr. 70° C.; 24 hr. 37° C. ....	77.4	58.5	125.9	112.8	96.2	117.9	144.6
48 .....	74.5	41.5	129.4	132.0	125.3	117.9	132.1
120 .....	52.8	33.3	154.3	159.2	235.4	135.7	158.0
FRENCH ROOTS							
24 .....	79.7	52.7	124.2	124.1	96.9	107.7	134.1
1 hr. 70° C.; 24 hr. 37° C. ....	92.6	45.7	108.9	103.7	80.0	101.9	119.3
48 .....	73.0	49.6	132.3	136.1	103.1	119.2	135.2
120 .....	69.9	34.9	135.9	140.7	164.6	119.2	139.8
OHIO CROWNS							
24 .....	90.3	36.2	115.8	114.4	81.7	100.0	134.9
48 .....	82.6	28.4	128.3	129.7	125.0	106.1	149.2
FRENCH CROWNS							
24 .....	84.1	47.6	129.2	119.0	89.3	105.4	172.2
48 .....	79.4	38.4	137.8	133.0	112.5	114.6	170.4

**TABLE IX**  
**DISTRIBUTION OF NITROGEN IN RED CLOVER ROOTS AND CROWNS SAMPLED MARCH 25, 1935. RESULTS ARE EXPRESSED AS PERCENTAGE**  
**OF THE FRESH WEIGHT**

PERIOD OF AUTOLYSIS	TOTAL		PROTEIN		EXTRACTED		NON- PROTEIN		ALPHA- AMINO		BASIC		AMIDE		RESIDUAL	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
<i>Hours</i>																
OHIO ROOTS																
(Check) 0	0.452		0.257	0.091	0.195	0.082	0.083	0.033	0.080							
24	.....		0.228	0.051	0.224	0.089	0.075	0.032	0.103							
48	.....		0.174	0.042	0.278	0.115	0.103	0.041	0.122							
72	.....		0.174	0.040	0.278	0.116	0.145	0.039	0.123							
FRENCH ROOTS																
(Check) 0	0.405		0.246	0.080	0.159	0.060	0.068	0.025	0.074							
24	.....		0.187	0.049	0.218	0.084	0.085	0.031	0.103							
48	.....		0.181	0.038	0.224	0.090	0.089	0.034	0.100							
72	.....		0.184	0.037	0.221	0.089	0.129	0.031	0.101							
OHIO CROWNS																
(Check) 0	0.495		0.359	0.210	0.136	0.062	0.043	0.033	0.041							
24	.....		0.284	0.103	0.211	0.091	0.057	0.038	0.082							
48	.....		0.266	0.071	0.229	0.106	0.065	0.041	0.082							
72	.....		0.255	0.056	0.240	0.114	0.097	0.042	0.084							
FRENCH CROWNS																
(Check) 0	0.525		0.411	0.237	0.114	0.049	0.036	0.023	0.042							
24	.....		0.329	0.111	0.196	0.084	0.051	0.033	0.079							
48	.....		0.312	0.086	0.213	0.098	0.069	0.035	0.080							
72	.....		0.306	0.074	0.219	0.103	0.088	0.036	0.080							

**TABLE X**  
**DISTRIBUTION OF NITROGEN IN RED CLOVER ROOTS AND CROWNS SAMPLED MARCH 25, 1935. RESULTS ARE EXPRESSED AS PERCENTAGE OF THE ORIGINAL, TAKEN AS 100 PER CENT.**

PERIOD OF AUTOLYSIS	PROTEIN		EXTRACTED PROTEIN		NON-PROTEIN		ALPHA-AMINO		BASIC		AMIDE		RESIDUAL	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Hours	OHIO ROOTS													
24	88.7		56.0		114.9		108.5		90.4		97.0		128.8	
48	67.7		46.2		142.6		140.2		124.1		124.2		152.5	
72	67.7		44.0		142.6		141.5		174.7		118.2		153.8	
FRENCH ROOTS														
24	76.0		61.2		137.1		140.0		125.0		124.0		122.6	
48	73.6		47.5		140.9		150.0		130.9		136.0		119.0	
72	74.8		46.2		139.0		148.3		189.7		124.0		120.2	
OHIO CROWNS														
24	79.1		49.0		155.2		146.8		132.6		115.2		200.0	
48	74.1		33.8		168.4		171.0		151.2		124.2		200.0	
72	71.0		26.7		176.5		183.9		225.6		127.3		204.9	
FRENCH CROWNS														
24	80.0		46.8		171.9		171.4		141.7		143.5		188.1	
48	75.9		36.3		186.8		200.0		191.7		152.2		190.5	
72	74.5		31.2		192.1		210.2		244.4		156.5		190.5	



In the clover crown tissue, proteolysis continued up to 72 hours. As in January, greater percentage increases in non-protein nitrogen were observed in the French crowns than in the Ohio crowns. The decrease in extracted protein again was greater than the increase in non-protein nitrogen.

TABLE XI

NON-PROTEIN NITROGEN IN RED CLOVER ROOTS AND CROWNS EXPRESSED AS PERCENTAGE OF THE TOTAL NITROGEN

SAMPLED JANUARY 30, 1935		SAMPLED MARCH 25, 1935	
PERIOD OF AUTOLYSIS	NON-PROTEIN N	PERIOD OF AUTOLYSIS	NON-PROTEIN N
<i>Hours</i>	%	<i>Hours</i>	%
	OHIO ROOTS		OHIO ROOTS
(Check) 0 .....	46.51	(Check) 0 .....	43.14
24 .....	60.79	24 .....	49.56
1 hr. 70° C.; 24 hr. 37° C. ....	58.57	48 .....	61.50
48 .....	60.16	72 .....	61.50
120 .....	71.75		
	FRENCH ROOTS		FRENCH ROOTS
(Check) 0 .....	45.59	(Check) 0 .....	39.26
24 .....	56.62	24 .....	53.83
1 hr. 70° C.; 24 hr. 37° C. ....	49.63	48 .....	55.31
48 .....	60.29	72 .....	54.57
120 .....	61.95		
	OHIO CROWNS		OHIO CROWNS
(Check) 0 .....	38.06	(Check) 0 .....	27.47
24 .....	44.07	24 .....	42.63
48 .....	48.84	48 .....	46.26
		72 .....	48.48
	FRENCH CROWNS		FRENCH CROWNS
(Check) 0 .....	35.24	(Check) 0 .....	21.71
24 .....	45.53	24 .....	37.33
48 .....	48.57	48 .....	40.57
		72 .....	41.71

A comparison of relative enzymatic activity in January and in March is furnished by calculation of the non-protein nitrogen as percentages of the total nitrogen of the root and crown tissue. These data are shown in table XI. Autolysis of the roots for 120 hours in January resulted in hydrolysis of protein to the extent of more than 25 per cent. of the total nitrogen of the Ohio roots and but 16 per cent. of the total nitrogen of the French roots. In March the corresponding figures were 18 per cent. for the Ohio roots and 15 per cent. for the French roots during an autolysis period of 72 hours.

TABLE XII  
DISTRIBUTION OF NITROGEN IN RED CLOVER ROOTS

SAMPLING DATE	VARIETY	STORAGE TEMP.	TOTAL		PROTEIN		EXTRACTED PROTEIN		NON-PROTEIN		ALPHA-AMINO		AMIDE		RESIDUAL N	
			N	%	N	%	N	%	N	%	N	%	N	%	N	%
1936		°F.	PERCENTAGE OF FRESH WEIGHT													
Feb. 11	Ohio	34 (check)	0.456	0.260	0.126	0.196	0.096	0.045	0.055							
" "	French	34 (check)	0.452	0.256	0.137	0.196	0.089	0.041	0.066							
Feb. 22	Ohio	34	0.426	0.216	0.140	0.210	0.095	0.041	0.074							
" "	French	34	0.377	0.228	0.125	0.149	0.067	0.033	0.049							
" "	Ohio	31	0.470	0.254	0.121	0.216	0.097	0.043	0.076							
" "	French	31	0.501	0.288	0.157	0.213	0.092	0.044	0.077							
" "	Ohio	12	0.589	0.402	0.125	0.187	0.084	0.039	0.064							
" "	French	12	0.518	0.389	0.100	0.129	0.055	0.025	0.049							
PERCENTAGE OF DRY WEIGHT																
Feb. 11	Ohio	34 (check)	2.840	1.618	0.786	1.222	0.598	0.281	0.343							
" "	French	34 (check)	3.178	1.800	0.963	1.378	0.626	0.288	0.464							
Feb. 22	Ohio	34	2.969	1.507	0.975	1.462	0.514	0.253	0.376							
" "	French	34	2.890	1.747	0.959	1.143	0.609	0.270	0.478							
" "	Ohio	31	2.950	1.593	0.760	1.357	0.560	0.268	0.468							
" "	French	31	3.050	1.754	0.956	1.296	0.402	0.187	0.305							
" "	Ohio	12	2.819	1.925	0.598	0.894	0.402	0.187	0.305							
" "	French	12	2.922	2.120	0.544	0.702	0.299	0.136	0.267							
PERCENTAGE OF TOTAL NITROGEN																
Feb. 11	Ohio	34 (check)	57.02	27.63	42.98	21.05	9.87	12.06								
" "	French	34 (check)	56.64	30.31	43.36	19.69	9.07	14.60								
Feb. 22	Ohio	34	50.70	32.86	49.30	22.30	9.62	17.38								
" "	French	34	60.48	33.16	39.52	17.77	9.15	13.00								
" "	Ohio	31	54.04	25.74	45.96	20.64	9.15	16.17								
" "	French	31	57.49	31.34	42.51	18.36	8.78	15.37								
" "	Ohio	12	68.25	21.22	31.75	14.26	6.62	10.87								
" "	French	12	75.10	19.31	24.90	10.62	4.83	9.45								

In January slightly less than 11 per cent. of the total nitrogen in the Ohio crowns was hydrolyzed in 48 hours. For the same period more than 13 per cent. of the total nitrogen in the French crowns was hydrolyzed. An autolysis period of 48 hours in March brought about hydrolysis of nearly 19 per cent. of the total nitrogen in both Ohio and French crowns.

The distribution of nitrogen in red clover roots stored for 11 days in darkness at low temperatures is shown in table XII. In this experiment the plants were undisturbed until sampled for analysis. Regardless of storage temperature the Ohio roots maintained a larger proportion of their total nitrogen as non-protein nitrogen than did the French roots. In the autolysis studies the Ohio roots were always higher in non-protein nitrogen than were the French roots. Proteolysis occurred in the Ohio roots stored at 34° F. or 31° F. A storage temperature of 12° F. resulted in a marked decrease in the proportion of non-protein to total nitrogen as compared with the original or check analysis. Even greater decreases were noted in the French roots stored at 12° F. To a lesser extent the same decrease was observed in French roots stored at 31° or 34° F. It is not possible with the available data to explain these decreases in non-protein nitrogen.

### Discussion

The hardier variety (Ohio) contains a larger percentage of sugars than the less hardy (French) under cold-hardened conditions. A further interesting question is whether this greater quantity of sugar is due primarily to greater conversion of starch and dextrins to sugar, or whether the hardier variety is instead more conservative of the sugar already formed. This investigation indicates that the hardier clover is more conservative of its already formed sugar, as can be noted from tables I to VI. NEWTON and ANDERSON (6) have established varietal differences in wheat plants by means of their metabolic rates at temperatures below 0° C. A similar relationship seems to exist between hardy and less hardy varieties of red clover.

It is very difficult to learn to what extent enzymatic hydrolysis of the carbohydrates in macerated red clover roots and crowns can be correlated with naturally occurring enzymatic activity in plants grown under controlled or field conditions. The data of tables V and VI show that the changes that occurred in the roots of the undisturbed clover plants when placed in darkness at temperatures of 34°, 31°, and 12° F. were similar to those in macerated tissue (autolysis). In a previous publication (3) it was reported that the Ohio variety of red clover grown under field conditions maintained a higher level of sugars and starch than the French variety. The present investigation shows that the enzymes active on starch in the French variety were capable of hydrolyzing more starch in a given length of time than were those in the Ohio variety. Thus, it would appear that the

carbohydrate enzymatic activity in macerated clover tissue was similar to that naturally occurring in plants under field conditions.

There is a difference in the protective action between the two varieties of red clover. The hardy variety showed a greater protective action than the less hardy variety, after heating the tissue for 1 hour at 70° C. Exposure of the tissue to -22° C. for 1 hour did not influence the enzymatic activity when compared with the check. A number of investigators have attempted to correlate total sugar, amino acids, inorganic salts, pH, etc., and protective action to heat and cold by the addition of these substances to plant saps. This investigation shows a greater protective action with a greater quantity of sugar and non-protein nitrogen. A critical study of the percentages of these substances in the tissue will reveal the fact that any one factor such as sugar, etc., is not responsible for the difference in protective action, of the tissue at 70° C. for 1 hour. Undoubtedly, the ability of the enzyme complex to withstand heat is a test directly or indirectly of the stability of the entire cell-constituent complex.

In a previous publication (3) it was reported that, with the approach of winter, French red clover roots increased in non-protein nitrogen more rapidly than did Ohio roots. Whether this increase in non-protein nitrogen occurred through proteolysis or simply by accumulation of soluble nitrogen not yet organized into protein cannot be determined. The present investigation shows that the proteolytic enzymes in the Ohio clover roots were capable of hydrolyzing more protein in a given length of time than were those in the French clover roots.

Similarly under field conditions proteolysis was more rapid in the Ohio crowns than in the French crowns (3). Yet in the autolysis experiment greater proteolysis occurred in the French crowns than in the Ohio crowns.

It is considered that a high level of sugars in plants helps to prevent protein precipitation. The writers have shown (2, 3) that the Ohio variety of red clover is nearly always higher than the French variety in sugars and starch. At the same time the French variety usually maintains the higher proportion of its total nitrogen in the form of protein.

It is quite possible that the analytical determinations designed as indices of cold-hardiness are not of equal value for all plant species.

### Summary and conclusions

1. The results of this investigation suggest that the adaptability of the red clover plant to winter conditions is closely associated with its rate of carbohydrate metabolism. The enzymes active on starch in the French variety (less hardy) were capable of hydrolyzing a greater proportion of the starch in a given length of time than were those in the Ohio variety. The proteolytic enzymes in the Ohio clover roots were more active than were those

in the French clover roots. A reverse relationship existed for the proteolytic enzymes in the crown tissue.

2. Heating the macerated clover tissue to 70° C. for 1 hour did not destroy the carbohydrate and proteolytic enzymes, although accumulation of sugars and non-protein nitrogen was not so great as in the untreated samples. The enzymes in the French variety of red clover were injured more than those in the Ohio variety by the heat treatment. This protected enzymatic activity is correlated with larger percentages of sugar and amino nitrogen in the Ohio variety than in the French variety.

3. Exposure of the tissue to -22° C. for 1 hour previous to autolysis did not greatly influence the enzymatic activity, when compared with the check.

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# WATER ABSORPTION IN THE COTTON PLANT AS AFFECTED BY SOIL AND WATER TEMPERATURES<sup>1</sup>

C. H. ARNDT

## Introduction

In experiments at the South Carolina Experiment Station with solution cultures of cotton (*Gossypium hirsutum* L.) it was occasionally noted in early spring that severe wilting occurred in the morning when the greenhouse temperature had fallen as low as 16° C. during the preceding night. This unusual wilting was apparently due to sunlight causing a rapid rise in air temperature when the solution temperature was low; for the plants recovered quickly when the air temperature was lowered by ventilation until the solutions had become warmed to about 20° C. Similar morning wilting was observed in plants growing in soil when soil temperature was much lower than a rapidly rising air temperature. Because the relation of root absorption to soil temperature is of great importance in the study of plant-water relations and also because the soil and solution temperatures which led to the wiltings just mentioned are higher than those reported for other plants, a study was undertaken to obtain information concerning the relation of root temperature to wilting in the cotton plant under otherwise favorable conditions for its healthy functioning. Some results of that study are briefly set forth in this paper, in the preparation of which the author has been aided by Professor BURTON E. LIVINGSTON of the Johns Hopkins University.

## Materials and methods

Observations were made on plants growing in solution and soil cultures. In the latter, young, vigorous, six-week-old plants were used; these had been grown in sheet-metal cylinders (25 cm. in diameter and 30 cm. deep), containing 18 kg. of a fertile sandy-loam soil. The average water content of the soil was held at 60 per cent. of the water-holding capacity, the water loss being replaced semi-weekly. To cool the soil, the cylinders were set in a tank of colder water, the water surface being about 3 cm. above the level of the free soil surface. The water temperature was maintained at approximately 1° below the desired soil temperature by means of an electric automatic water heater and a manually controlled stream of cold water from a refrigerating machine. When the desired soil temperature had been reached, the water bath was raised to that temperature. When lowering of soil temperature was not over 10°, approximately uniform soil tempera-

<sup>1</sup> Technical contribution no. 46 (new series), South Carolina Agricultural Experiment Station.

tures could be established throughout the soil mass in 5 hours. The soil temperature was measured at a depth of 4 cm. in the axis of the cylinder; if the temperature of parts of the root system differed from this, it was undoubtedly always slightly lower rather than higher, and the total difference was never more than one or two degrees.

In the solution-culture studies, 8-week-old plants, averaging 60 cm. in height, were used. These were well branched and leafy, with numerous floral buds and even young bolls. Two-liter Erlenmeyer flasks were used as containers, a single plant in each. The solution employed, which had been found quite satisfactory for the cotton plant, had the following composition:  $\text{KNO}_3$ , 0.0014*M*;  $\text{Ca}(\text{NO}_3)_2$ , 0.0028*M*;  $\text{KH}_2\text{PO}_4$ , 0.0007*M*; and  $\text{MgSO}_4$ , 0.001*M*; ferric citrate, 0.00001*M*;  $\text{MnSO}_4$  and  $\text{Na}_2\text{B}_4\text{O}_7$ , each 0.00002*M*; and  $\text{CuSO}_4$ , 0.0000005*M*. The solutions were renewed semi-weekly, water being added as needed to maintain a solution volume of 2 liters. To prevent excessive heating of the solutions by sunlight, each flask was placed in a 9-liter stone-ware jar, with excelsior packing, both jar and flask being covered with a cone of thick paper with a central opening for the plant. During the growth period the solution temperature showed a daily range of approximately 20°–35°. The solution temperature was lowered by a procedure similar to that employed for the soil cultures. During the cooling, heat transfer was accelerated by slowly bubbling air through the solution and by an occasional shaking. In other experiments cooling was accelerated by replacing the solution with a fresh one at the desired temperature before placing the flask in the water bath.

When roots were to be heated, a large cylindrical water bath was brought to the desired temperature before the plant container was suspended in it. This temperature was maintained throughout the experiment by means of a manually controlled electric heater. The container fitted closely into the cover of the bath and a large circular sheet of cardboard was fitted closely around the base of the plant, to prevent heated air from rising around the plant.

Air temperature and humidity were recorded by means of a shaded hygrothermograph adjacent to the plants on which the observations were being made.

### Experimentation

Unless otherwise noted, all experiments here described were performed in full sunlight in an unshaded greenhouse between April 1 and August 25, 1933. Wilting of the plants first became evident as a loss of turgidity of the leaves and a drooping of their margins. At a more advanced stage (recorded as severe wilting) there was noticeable drooping of the petioles and ends of the branches. During the heat-treatments the areas between the veins usually became noticeably bleached. These bleached areas disappeared on

cooling the roots, only to reappear several days later as yellow to brown areas. Such affected leaves usually dropped from the plant within a week.

#### WILTING INDUCED BY SUDDEN LOWERING OF SOLUTION TEMPERATURE

In twelve tests in which the temperature of the root system was suddenly lowered by replacing the old solution with a new and colder one, the latter being from 10° to 23° colder than the former, wilting began within 20 to 75 minutes. The length of this period showed no consistent relation to (a) the solution temperature at which wilting occurred (which varied from 11° to 16°), to (b) the initial solution temperature (which varied from 28° to 36°), to (c) the temperature lowering to which the roots were subjected (which varied from 10° to 23°), to (d) the current air temperature (which varied from 32° to 36°), to (e) the difference between root and current air temperature (which varied from 17° to 24°), or to (f) the current relative humidity (which varied from 25 to 50 per cent.). The following examples illustrate the actual results of specific experiments:

(1) Solution temperature lowered from 28° to 14°; wilting observed in 20 min., at air temperature 38°, relative humidity 35 per cent.; solution was then allowed to become warmer and plant had fully recovered in 85 min., solution temperature then being 22°.

(2) Solution temperature lowered from 33° to 11°; wilting observed in 17 min., at air temperature 35°, relative humidity 45 per cent.; leaves flaccid and petioles drooped after additional 15 min.; plant fully recovered 3 hr. after removal from the cold bath when solution temperature had risen to 25°.

(3) Solution temperature lowered from 30° to 15°; wilting observed in 45 min., at air temperature 34°, relative humidity 50 per cent.; solution then allowed to become warmer, plant fully recovered in 45 min., when solution temperature had risen to 22.5°.

#### WILTING INDUCED BY GRADUAL LOWERING OF SOLUTION TEMPERATURE

When the temperature of the roots was lowered gradually by immersing the flask in the cold bath without changing the solution, the processes leading to wilting went on concurrently with cooling, which was usually still in progress when wilting was first observed; at this time record was made of solution temperature and of the time elapsed since cooling began (which is here the same as the wilting time). In eleven tests in which initial solution temperature varied from 20° to 32° and time of cooling varied from 30 to 120 min. (with air temperature ranging from 30° to 39° and relative humidity from 20 to 50 per cent.), the plants wilted at various temperatures between 10° and 18.5°. There were no apparent relations among: (a) temperature difference between solution and air at time of wilting, (b) extent of cooling, and (c) wilting temperature; but larger solution-air differences generally



corresponded to more extensive cooling and to lower wilting temperatures. Conversely, none of the three variables just mentioned showed any consistent relation either to (d) wilting (cooling) time or to (e) rate of cooling, but the latter (d and e) were clearly related to each other; *i.e.*, when the rate of cooling was rapid the wilting time was short, and conversely, as might be expected. It may be said in general that, within the frame of these experiments, wilting began at solution temperatures between  $10^{\circ}$  and  $18.5^{\circ}$  and that it occurred more promptly when root temperature was lowered rapidly than when cooling was more gradual. The following detailed accounts are representative of the tests with gradually cooled solutions:

(1) Initial solution temperature  $21^{\circ}$ ; wilting began after 30 min., at solution temperature  $16.5^{\circ}$ ; air temperature  $32^{\circ}$  and relative humidity 35 per cent. at the beginning of visible wilting.

(2) Initial solution temperature  $21^{\circ}$ ; solution cooled for 105 min., when wilting began, at solution temperature  $15.5^{\circ}$ ; air temperature at beginning of visible wilting  $35^{\circ}$ , relative humidity 25 per cent.

(3) Initial solution temperature  $30^{\circ}$ ; solution cooled for 30 min., when wilting began (11:15 A.M.), at solution temperature  $18.5^{\circ}$ ; air temperature at beginning of wilting  $38^{\circ}$ , relative humidity 20 per cent. Solution was then allowed to warm to air temperature and recovery was complete before 2:45 P.M., when air temperature was  $29.5^{\circ}$ ; solution was then cooled until it reached a temperature of  $11.5^{\circ}$ , after 110 min. (4:35 P.M.) but plant did not wilt; air temperature at this time  $35^{\circ}$ , relative humidity 40 per cent.; sky had been overcast since 2 P.M. Failure to wilt at this low solution temperature was apparently related to cloudiness (see below, effect of shading soil cultures at low soil temperature), decreased air temperature, and increased relative humidity.

(4) Initial solution temperature  $25^{\circ}$ ; solution cooled for 40 min., when wilting began, at solution temperature  $10^{\circ}$ ; air temperature at this time  $35^{\circ}$ , relative humidity 50 per cent. Solution was then held at  $10^{\circ}$  for 4 hours longer, at the end of which period (late afternoon) all leaves were flaccid and petioles and ends of branches drooped. Plant regained turgidity the following night with solution at the temperature of the greenhouse, but on the following morning some of the younger leaves were observed to be slightly blackened on their margins. Several days later areas between the veins of these same leaves and of some of the other young leaves, which had not yet reached their full size, became somewhat yellowish and remained so for several days. Subsequent growth was apparently normal, excepting that many young roots had been injured. These had lost their geotropic sensitiveness and elongated at right angles to the pull of gravity; they became somewhat hooked at the ends and each showed a marked constriction behind the tip. The injured roots elongated only slightly, but many healthy branches developed from their older portions.

## WILTING INDUCED BY LOWERING OF SOIL TEMPERATURES

In studying the influence of unusually low soil temperatures on infection of cotton seedlings, wilting of healthy plants had been observed on clear days with high greenhouse temperature and low relative humidity at times when soil temperature was about  $18^{\circ}$  or lower. The soil temperature at which such wilting occurred was apparently always essentially the same whether the plants had been grown at constant soil temperatures ( $18^{\circ}$ ,  $21^{\circ}$ ,  $24^{\circ}$ ,  $27^{\circ}$ ,  $30^{\circ}$ ), or had been subjected to the daily temperature fluctuations of the greenhouse, or had been subjected to artificially controlled semi-daily temperature alternations ( $30^{\circ}$  by day and  $16^{\circ}$  by night); also whether the holard at the time of wilting represented 80 per cent. of the water-holding capacity of the soil, or only 60 per cent. This wilting was observed, however, only in full sunlight; it was never observed on cloudy days, even when soil temperature was below  $18^{\circ}$  and air temperature was relatively high. Furthermore, plants that had wilted in this way on clear days regularly exhibited rapid recovery in the late afternoon when the intensity of solar radiation was rapidly diminishing.

Some special experiments were carried out concerning the effect of shading on the occurrence of wilting. Plants grown in the usual soil containers were arranged in pairs in the same cold water bath; one of each pair was left in full sunlight, while the other was shaded from direct sunlight by a 60-cm. square of thick cardboard supported horizontally 20 cm. above the plant, so that air circulation around the plant would not be greatly modified. These tests were made on clear days in July. The unshaded plants wilted more or less promptly at a soil temperature of  $18^{\circ}$ , but none of the shaded plants wilted. It therefore appears that intense sunshine and low soil temperature acted in conjunction to produce wilting. The following accounts are representative of these tests with shaded and unshaded plants.

(1) At 8:30 A.M. on July 23 a pair of similar plants were placed in the cold water bath (temperature  $16^{\circ}$ ), where they remained until late in the afternoon of the next day. At 12:15 P.M. the unshaded plant was very noticeably wilted but the shaded one showed no wilting. Soil temperature was then  $18^{\circ}$  in the central axis,  $16^{\circ}$  at the margin, air temperature was  $36^{\circ}$  and relative humidity was 45 per cent. At 3:00 P.M. (air temperature  $37^{\circ}$ , relative humidity 35 per cent., soil temperature as above) the shaded plant was still unwilted but wilting of the unshaded plant was more pronounced than at 12:15 P.M. (At this time it was observed that similar cultures on a nearby greenhouse bench—unshaded and with soil temperature  $41^{\circ}$ —showed no wilting.) During the night the wilted plant recovered and appeared perfectly healthy at 8:30 A.M. the following morning, when soil temperature in the central axis was  $17^{\circ}$ , air temperature and relative humidity being  $30^{\circ}$  and 60 per cent. The leaves of the unshaded plant became gradually more

flaccid until 2:30 P.M., when the shade was removed. At that time (air temperature 36°, relative humidity 35 per cent.) the shaded plant remained apparently unaffected. At 4:45 P.M. both plants were severely wilted.

(2) July 7, 2:15 P.M., cultures with soil temperature 32° were placed in water baths at 18°, 20°, 22°, and 29°. At 5:00 P.M. (air temperature 38°, relative humidity 20 per cent.), when the soil masses had reached approximately the same temperatures as the baths, the plants in the 22° and the 29° baths were unwilted; those in the 20° bath (soil 22°) were slightly wilted; while those in the 18° bath (soil 20°) were badly wilted. At 8:30 A.M. the following day (air temperature 25°, relative humidity 55 per cent.), none of these plants was wilted, although the soil in the 18° bath had been cooled to 15° by cutting off the electric heater and increasing the supply of cold water. Because of inadequate cold-water supply, the temperature of that bath rose slowly and at 11:00 A.M. the soil had reached a temperature of 18° and the plants were slightly wilted (air temperature 33°, relative humidity 25 per cent.). At 3:00 P.M. these same plants were severely wilted (petioles drooping, youngest leaves more wilted than old ones), although the soil had reached a temperature of 20° at a depth of 4 cm. in central axis (air temperature 39°, relative humidity 15 per cent.). The plants in the 22° soil were unwilted. All wilted plants recovered quickly after sunset, even in the coldest bath, in which the soil temperature had again fallen to 18°.

(3) July 16, 10:00 A.M., six cultures were placed in water bath at 20°; at 2:00 P.M., with soil temperature approximately like that of bath, all plants were unwilted (air temperature 36°, relative humidity 20 per cent.). Three of these plants were then removed to a 17° bath. At 4:20 P.M. (air temperature 37°, relative humidity 20 per cent.) all plants in the 17° bath (soil 18°) were severely wilted, but those in the 20° bath (soil 20°) still showed no signs of wilting.

(4) July 15, 10:00 A.M., several cultures with soil temperature 22° were placed in bath at 16°. At 10:40 A.M. plants were severely wilted (soil temperature at 4 cm. depth 16° in center and 19° near margin; air temperature 32°, relative humidity 28 per cent.). Cultures were then removed to greenhouse bench and plants had completely recovered 35 min. later, at which time soil temperature near margin of cylinder had risen to 20° (air temperature and relative humidity approximately the same as at 10:40 A.M.).

In full sunlight, with low relative humidity and air temperature 30° to 40°, the plants generally wilted at soil temperatures from 16° to 20°, the higher soil temperatures being generally associated with the higher air temperatures and lower humidities. Thus the plants growing in soil wilted at slightly higher temperatures than those growing in solution cultures.

WILTING AND OTHER INJURIES INDUCED BY RAISING SOLUTION  
TEMPERATURE TO 60° C. OR HIGHER

Several earlier studies, which have been reviewed by KRAMER (12), and to which he has added confirmatory observations on cotton and several other plants, have shown that plants, whose roots have been killed by high temperatures, may remain unwilted for several days. Some of the writer's results from experiments with heated roots are in essential agreement with the earlier observations, but some additional effects of the temporary application of high temperature to cotton roots are to be noted.

In routine solution cultures of cotton grown in the greenhouse, the solution temperature frequently reached 40° and remained so for several hours on bright sunny days with no apparent injury to either tops or roots. In special experiments, heating the solution for 75 min. at 50° produced no noticeable injury and no reduction in the rate of transpiration. Prompt wilting and subsequent additional symptoms of injury were produced, however, when the solution around the roots was heated to 70° during a 75-min. period and then held at that temperature for 15 min. For the first day after this treatment, a plant thus treated transpired at about the same rate as the unheated controls; but for the second day and the five succeeding days, its daily transpiration rates were respectively only 14, 31, 38, 19, 23, and 14 per cent. of the corresponding control rates. The effect of this heat treatment on transpiration was thus not immediate, but it became evident after 24 hours. The actual water loss after treatment, however, was considerable, being (for the 7 days in order) 115, 30, 60, 110, 50, 50, and 30 gm. On the last day of this record the transpiration for the control plant was 220 gm., or about seven times as great as that of the heated plant. Similar results were secured with respect to transpiration when the solutions were heated to 60°, except that the retardation of transpiration was more gradual and longer delayed; *e.g.*, a plant whose solution had been heated to 60° in 30 min. and kept at that temperature for 75 min. gave the following successive rates of water loss (expressed as percentages of the corresponding control rates): 95, 93, 37, 24, 19, 14 gm. Thus notable retardation was not noticeable until the third day, after which it became gradually more pronounced until both the 60°- and 70°-treated plants showed the same degree of retardation. These observations suggest that, although these heat treatments killed or greatly injured the roots, the latter were able to supply water to the rest of the plant at an adequate rate for a day or more after the treatment—the length of this period being shorter with the more severe treatment.

Additional symptoms of injury that accompanied or followed these heat treatments were readily observed. When the solutions were heated to 60° or higher, numerous gas bubbles escaped from the root lenticels while heating was in process, apparently because of gas expansion in intercellular

spaces. Also, wilting of the older leaves occurred while the 70° treatment was in progress, but wilting did not occur with the 60° treatments. The 70°-treated plants recovered from wilting during the night and were apparently healthy the following day. Concurrently, however, with the reduction of transpiration on the second day after treatment, wilting of the older leaves again became apparent, areas between the veins became discolored, and there was no subsequent recovery. All plants subjected to solution temperature of 60°, or higher, for a period of 60 min. showed conspicuous light-colored areas between the veins of the older leaves before the end of the heating period. After the solution or soil had cooled to air temperature these areas disappeared, but they reappeared a day or two later as conspicuous yellow or yellow-brown areas. Leaves so affected became abnormally rigid and leathery, never recovered, and eventually fell from the plant. The larger veins of the leaves of the 60°-treated plants retained their bright green color for several days after the discoloration of neighboring areas. Before a leaf dropped these veins often became darker brown than the rest of the leaf, appearing much like veins discolored through leaf infection by *Phytophthora malvacearum*. All such leaves were shed from the 70°-treated plant within a week; but they were retained somewhat longer (8–10 days) on the plants whose roots had been subjected to the 60° treatment. The youngest leaves showed no discolored areas and were retained longest. Some flowers opened quite normally, even after all but the very youngest leaves near the stem tip had fallen.

Roots that received the 70° treatment made no real growth subsequent to the treatment, although some of the tips enlarged noticeably, became bulbous, and retained their usual color for several days. All of them died and became brown within a week. Following the 60° treatment, some elongation of roots continued for several days; but the new root region formed was irregular in diameter and its apical portion tended to become bulbous, as when roots had been subjected to 10° for 4 hours. The older portions of the roots seemed to have been more severely affected by the treatment; they became dark brown and were apparently dead within a few days. After two weeks all roots were flaccid, gelatinous, and unquestionably dead.

Sections cut a week after the most severe treatment (70°) showed the xylem of the tap root to be dark brown, and brownish streaks were observed extending upward into the xylem of stem, branches, and petioles. Root cortex was obviously dead, but stem cortex was still green and showed no signs of injury. After a recovery period of three weeks, plants that had received the 60° treatment showed no discoloration of root xylem; but the root cortex was dead, excepting a small portion that had been above the level of the solution surface at the time of treatment. On the other hand, a brownish discoloration was evident in the xylem above the collet, in

streaks which were progressively less pronounced farther up. In a 60°-treated plant sectioned after 12 days, some discoloration was present even in the petioles. All leaves on the 60° plants at the time of treatment dropped before the end of three weeks. At that time there were some weak green shoots arising from the lower portion of the main stem.

#### INJURY INDUCED BY RAISING SOIL TEMPERATURE TO 60° C. OR HIGHER

Experiments in which the soil temperature was temporarily raised to 60° or 63° gave results similar to those obtained with the heat-treated solutions. On clear warm days, when the aerial surroundings were such as to produce relatively rapid transpiration, these heat treatments of plants in soil always produced pale or nearly white areas between the larger veins of the leaves while the treatment was in progress. More or less wilting usually occurred during the treatment; in several instances even the tips of the branches lost their turgidity. When the soil was cooled to greenhouse temperature immediately after the treatment, the discolored areas between the veins disappeared and the plants regained their turgidity. No sign of wilting or discoloration was present on the day after treatment, but after an additional day or two those leaves that had become severely discolored during treatment became flaccid and did not regain turgidity. All regions that had become discolored during treatment and had then apparently recovered became pale yellow again at this time, drying up and turning brown within several days. All such discolored leaves dropped from the plant within two weeks. Similar discolored areas appeared on leaves that had not wilted during treatment but had shown discoloration at that time. As in the solution cultures, these leaves soon became unusually rigid and leathery and later dropped from the plant.

A typical example may represent these experiments with heated soil. At 2:00 P.M., a healthy plant (soil temperature 40°, holard 60 per cent.) was transferred from the greenhouse bench to a 60° water bath. At 6:00 P.M. (soil temperature at the 4 cm. depth 56° in center and 65° near margin) the older leaves were severely wilted and there was a pronounced discoloration between veins in many unwilted leaves as well as in the wilted ones. The plant was then returned to the greenhouse bench, where it regained turgidity and the discolored areas disappeared within several hours. There were no visible abnormalities the day after the treatment. Most of the discolored areas, however, again became very conspicuous on the second day, reappearing as yellowish areas, which became dry and brown within a few days. Leaves, thus affected, fell within 10 days; but the young leaves on the plant at the time of treatment were still apparently healthy at the end of 3 weeks, during which period new leaves had formed at the tip of the main stem. On removing the plant from the soil at this time it was noted that all of the roots

present at the time of treatment were dead, that the xylem throughout the root system was brown, and that this discoloration extended upward in the stem xylem for 12 cm. In spite of this severe injury, new roots were arising from the collet region immediately below the surface of the soil and new branches were forming on the main stem.

### Discussion

References to the literature concerning the physiological effects of low soil temperatures may be found in the writings of KÖHNLEIN (10), WHITFIELD (17), PEIRCE (16), and CLEMENTS and MARTIN (6). It seems to be clear that wilting may be brought about, at least in a number of different plant forms, by a sufficient lowering of the root temperature in an adequately moist soil when transpiration is relatively rapid. CLEMENTS and MARTIN (6) induced visible wilting in sunflower at soil temperature of 4.5° (40° F.), but it required a still lower temperature to produce severe wilting. The only record of wilting at soil temperature above 4.5° appears to be that of KÖHNLEIN (10, p. 411), who noted partial wilting of sunflower at soil temperature of 17.5° or 18.5°, but the air temperature of his greenhouse was 42°; this is considerably higher than the air temperature prevailing when CLEMENTS and MARTIN noted wilting in the same plant and it is also considerably higher than the air temperature at which cotton plants wilted for the writer with soil temperature 20°. KÖHNLEIN also noted that his sunflower plants with soil at 18° wilted much more promptly than those with higher soil temperature in the same greenhouse.

It seemed remarkable that cotton plants wilted with root temperatures no lower than 18° under air conditions that were not unusually favorable for rapid transpiration, while they wilted at still higher root temperatures (20° to 21°) under air conditions that should have promoted unusually rapid transpiration. Different kinds of plants grown under similar conditions, and even plants of the same species grown under different environmental complexes, might be expected to wilt with different degrees of root cooling, even when all influential conditions except root temperature were quite healthful and alike at the time of wilting. It seems probable that, for any previously healthy individual plant, wilting induced by root cooling should occur with different degrees of cooling according to the combination of air temperature, air moisture, and radiation prevailing at the time of wilting. Such suppositions are based on the familiar consideration that wilting of this sort, which is immediately due to inadequacy of foliar moisture, is determined partly by the transpiration rate and partly by the rate at which water enters the leaves. In these root-cooling experiments the water-conducting capacity of the plants was surely not inadequate; neither was the environmental water supply inadequate, for the soil cultures were well supplied

with soil moisture and the water supply in the solution cultures was practically infinite. It therefore seems safe to suppose that these cold-root wiltings were occasioned by inadequacy in the capacity of the root systems to receive sufficient water from without and to permit its rapid movement into the conducting channels.

The capacity of a root system to receive water from the soil solution is of course dependent partly on the extent of the absorbing surfaces, partly on the rate of water movement from root to stem (essentially the current transpiration rate in such plants as cotton), and partly on the more or less obscure conditions that are effective within the root tissue between the absorbing periphery and the conducting channels. Lowering the root temperature, as in these experiments, surely could not have reduced the extent of the root surface, nor did it increase the transpiration rate. Consequently it appears that the cold treatment either reduced the capacity of the root tissue to receive water from an adequate external supply or else reduced its capacity to let water move into the vessels.

As to the manner in which the living cortex of the absorbing region of the root might possibly be markedly more resistant to water passage at 10° to 20° than at 30° to 40° a number of different hypotheses might be suggested, but to test them logically would require quantitative experimental data that are not yet available. Two possible hypotheses may be mentioned in a roughly qualitative way: (1) A very simple hypothesis may be based on the temperature relation of the viscosity of water, which is about one and one-half times as great at 20° as at 40°. At a specified pressure gradient water moves through finely porous material more rapidly as the temperature is raised. The pressure gradient by which water moved through the root cortex into the xylem in the experiments of this study could not have been increased, since negative pressure in plants becomes more pronounced with the approach of wilting. It follows that a reason for the slower water movement at lower than at higher root temperatures, as shown in these experiments, is to be sought in increased resistance rather than in decreased pressure gradient. According to this hypothesis such increased resistance is considered as due wholly to change in water viscosity; the colloidal structure and arrangements of non-water materials in the root being assumed as not effectively altered when root temperature is lowered. For some experimental findings concerning the influence of water viscosity on water movement through porous porcelain reference may be made to CHRISTIANSEN, VEIHMEYER and GIVAN (5). The observations of BODE (3) on the effect of temperature on the passage of water from soil through the roots of the sunflower to the stem and those of KRAMER (13) on the absorption of water from the soil by porcelain cones lend support to this hypothesis. (2) The temperature relations of solation and gelation in colloidal material might be



introduced as the basis for another qualitatively plausible hypothesis, if it is roughly supposed that the cortex cells interpose a greater resistance to water passage at lower than at higher temperature, not only because water is somewhat less fluid at lower temperatures but also because of changes in the ultramicroscopic arrangement, distribution, or configuration of their non-water material. Changes induced by lowering the temperature of the protoplasts may be readily supposed to render the entire tissue less permeable to water passage, as if the paths of water movement through them were narrowed or had become less numerous with decreasing temperature. Furthermore, it is at least logically possible that the ultramicroscopic porosity of the cell walls of the absorbing root cortex—including walls in immediate contact with the environmental water—may sometimes be reduced as the temperature is lowered.

It may be added that if the rate of water movement through the absorbing root cortex into the xylem (when the external water supply is adequate) is generally influenced by some sort of protoplasmic pumping action—as many writers have supposed—that action would be less effective as the water viscosity becomes greater and as permeability of the cortex and walls to water becomes less. Furthermore, the pumping action itself might be less vigorous at lower than at higher temperatures, which would be expected if such action really exists and if it is associated with respiratory activity—as suggested by LETA HENDERSON (8); for respiration is generally less rapid at lower than at higher temperature. But of course changes in cortex permeability—as those of solation and gelation—might be supposed to be greatly influenced by intensity of metabolic oxidation, whether pumping action is hypothesized or not. In this connection it is interesting to compare the soil temperature at which wilting occurred ( $20^{\circ}$  to  $21^{\circ}$ ) when conditions were favorable for rapid transpiration with the temperature relations of seed germination and subsequent growth in this tropical and subtropical species. CAMP and WALKER (4) and ARNDT (1) have observed that a soil temperature of about  $27^{\circ}$  was necessary for good top growth when air conditions were favorable, and FUNG (7) found that the optimal air temperature for top growth was  $30^{\circ}$  or higher. Some unpublished studies by the writer gave similar results. The writer has observed good root development at  $24^{\circ}$ , but then even at favorable air temperatures, top growth was retarded, as though the root systems were failing to supply the tops adequately with water or minerals, or both. Furthermore, germination percentage was found to be much reduced when the soil temperature was maintained at  $18^{\circ}$  to  $19^{\circ}$  (4, table IV; 1), which is about the same as the minimal soil temperature at which the cotyledons could be raised above the soil surface. With a soil temperature between  $18^{\circ}$  and  $20^{\circ}$  the cotyledons that did appear above the soil remained yellow for a week or longer, as though the roots were not sup-

plying requisite materials at an adequate rate. It is thus seen that the critical soil temperature for wilting of cotton in the cold treatments described in this paper is not greatly different from what has been found to be the minimal temperature for healthy seed germination and seedling development, while it is only slightly below the soil temperature necessary for healthy root growth.

The results here presented on the effects of lethal high temperatures when applied to previously healthy plants are in essential agreement with previous observations, as reported for cotton and other plants by KRAMER (12), but the present account adds observations on previously unreported wilting of leaves at the time of the treatment, also on the bleaching of regions between the veins while heat treatment was in progress, and on the subsequent history of these injured regions. Wilting was observed while the heat treatment was being applied (a) when the solution was heated to 70° and maintained at that temperature for 15 minutes, also (b) when the soil temperature was raised to 56° or 65° (four hours being required to reach 56° in the center of the soil container after it had been placed in a 65° water bath). That this wilting was not due to heat effect on water viscosity is obvious. It is also obvious that whatever caused this loss of leaf turgor ceased to be operative shortly after the root temperature was reduced to 36°, for the treated plants soon recovered. It appears, however, that the rate of movement of water through the cortex was, in those cases, temporarily lowered in some way during heat treatment. This may suggest a reversible change of some kind in the root tissues, but since there is little information available in this connection it would be premature to attempt hypotheses concerning the possible nature of such a change.

That killing most of the root system by heat did not immediately reduce transpiration considerably (and subsequently reduced it only slowly) renders it probable that dead or severely heat-injured roots were still sufficiently absorptive and sufficiently permeable to water to keep the foliage adequately supplied for a considerable period. If some kind of pumping action dependent on healthy protoplasm in the roots were necessary for maintaining the transpiration stream at an adequate rate it might be expected that wilting should occur promptly with the application of heat to the root system, without the maintenance of the original transpiration rate for a day or more thereafter. For a somewhat thorough discussion of a similar problem to the one here suggested, reference may be made to OVERTON (14, 15) and KRAMER (11, 12). It is clearly apparent that the vessels of the heat-treated plants did not become seriously plugged—as by gum deposits—during the heat treatments nor for at least a day or two thereafter. They may have become plugged still later. BERKLEY and BERKLEY (2) observed that water still passed upward rapidly through the stems and petioles of

their cotton plants after these organs had been killed by high air temperatures. Some time after the roots were killed their previously living cells must have collapsed completely, but their coarser structure was apparently maintained as long as the original transpiration rate was not greatly reduced. Otherwise, it is difficult to imagine them maintaining adequate capillary contact with the moisture films of the soil.

Discoloration of the areas of the older leaves while the roots were receiving the injurious or lethal heat treatment may not have been due to an inadequate water supply of the leaves. When cotton plants wilted from the effects of drought, discoloration did not regularly occur and did not appear in plants wilted by application of low temperatures to the roots; but the discoloration here described was not unlike the permanent bleaching that may be produced by exposing cotton leaves to lethal air temperature, nor is it unlike the discoloration (called "scald" by the cotton farmer) that appears at times on leaves of cotton plants infected with *Fusarium vasinfectum*. The leaf discoloration that accompanied heat treatment of the roots was superficially suggestive of poisoning. Some sort of mildly toxic material formed in the heat-injured cells of the roots may perhaps have been promptly carried to the lower leaves in the transpiration stream—or possibly in the phloem. If such a supposition is tenable it must be supposed further that the first mildly injurious effect in restricted regions of the older leaves was not lethal and was reversible, for the discolored areas between the veins vanished within a few hours after the heat treatment was discontinued—within 90 min. in one experiment, in which the leaves had not wilted. Perhaps if the heat treatments had been more severe or longer continued such spots would not have regained their normal green color. At all events it seems quite possible that this early discoloration was due to some toxic material that arose in or near the heated roots and was carried upward quickly, that its delivery in the leaves ceased or became much slower very soon after heating was discontinued, and that its localized injurious effects in the foliar tissue were reversible in rather prompt recovery.

The second appearance of localized foliar discoloration, in the same areas as those previously discolored, also resembled some discolorations produced by poisons—for example, that produced by toxic concentrations of  $\text{SO}_2$  on alfalfa in the experiments of HILL and THOMAS (9). This second discoloration sometimes first appeared as small, light brown spots (1 to 2 mm. in diameter), which rapidly coalesced to involve the entire area between adjacent large veins; the discoloration finally spread to the veins, as already noted. A similar discoloration of the leaves was noted on the cold-treated plants, but only when the temperature was lowered to  $10^\circ$ , which temperature was also sufficiently injurious to cause abnormal root development. In these latter plants, as contrasted to the heat-treated ones, the young leaves near the top

of the plant, which had not yet attained full development, were the first to show discoloration.

By the time of the second appearance of the brown areas on the leaves of plants whose roots had been heated, cell disintegration in the killed roots may have become greatly advanced and toxic material arising therein may have been of a more virulent type or more plentiful, or both, than during the short period of the heat treatment. The upward movement of such material might continue indefinitely, soon leading to local necroses in the affected leaves and to their eventual death. If these suppositions were approximately correct, it might be said, following OVERTON (15), of the affected regions and finally of these entire leaves, that they dried out because of death rather than that they died because of an inadequate water supply. It is, of course, not necessary to suppose the toxic material of the first evanescent discoloration during the period of heat treatment to have been at all of the same sort as that involved in the subsequent effects. It might be expected that the later injury should appear most promptly in tissues of localized regions that had been previously slightly affected and had apparently recovered; perhaps those tissues were originally unusually susceptible to injury of this sort and their recovery from the first poisoning may have been incomplete.

The brown stain, which soon appeared in the xylem of roots, stems, and even petioles of the heat-treated plants and its greater intensity and upward extent as the heat treatment was made more severe, may be taken as evidence that unusual material of some sort had passed upward from roots to foliage, for such stain has not been found in healthy plants. Discoloration immediately above the collet (*i.e.*, just above the most severely treated region) was usually darker than in the roots. It may be supposed that soluble substances not usually present in the transpiration stream were carried upward from the injured root systems, that some of these produced stain in the xylem vessels—perhaps also toxic effects in living cells of that tissue—and that some of them were responsible, first for localized injury in the leaves and then for the death of foliar tissue.

In connection with these hypothetical considerations concerning possible effects of toxic emanations from roots that had been killed or severely injured by heat treatment, some special attention should be given to the clearly differentiated but somewhat similar symptoms of leaf injury that followed excessive cooling of roots in these studies. Those symptoms were confined to the younger leaves, near the top of the plant, while it was the older leaves that were injured by excessive heating of roots. When the cold treatments were severe enough to cause abnormal root development (approximately 10°), the younger leaves were apt to show narrow regions of killed tissue at their margins on the day following the treatment and several days later

yellowish areas between the veins. There was no recovery of the marginal tissues, but the yellow areas between veins shortly disappeared, not leading to defoliation. The marginal injuries suggest effects of water deficiency and they might be regarded as analogous to tip burn. But the evanescent discoloration following cold treatment of the roots can hardly be attributed solely to excessive water deficit in localized regions, for such discoloration of young leaves is not commonly observed when plants are injured by ordinary drought when in the field. Perhaps these discolorations may indicate some form of mild poisoning, and it is at least logically possible to relate them tentatively to local accumulations—enhanced by foliar water deficit and transpiration—of toxic materials emanating from the injured roots. It will be recalled that these cold-treated plants exhibited some injury at the root tips and that the subsequent growth of many tips indicated physiological disturbance such as might result from disturbed metabolism and resultant toxic action.

### Summary

1. Cotton plants growing in the greenhouse wilted on clear days when the temperature of the roots was sufficiently lowered for a short time. Solution cultures showed this wilting when their temperature had been lowered to between 10° and 18° C.; for soil cultures, with ample water supply, the highest soil temperature at which wilting occurred was generally somewhat higher, between 17° and 20°. The amount of this lowering necessary to produce wilting varied, being clearly dependent on air temperature, relative humidity, and sunlight intensity.

2. Exposure of the roots of plants growing in solution or soil cultures to a temperature of 60° for 60 min. severely injured the roots and caused a marked reduction in the absorption of water by such plants. Plants in solution cultures did not wilt while being heated to 60° in 60 min. A temperature of 70° maintained for 15 min., however, did cause similar plants to wilt. Plants growing in soil wilted at a soil temperature of 60°. White areas appeared between the veins of the leaves during the heat treatments at 60° and higher. These discolored areas disappeared within several hours after the reduction of the soil temperature, only to reappear several days later as yellow or brown regions in which the tissues became dry and died. Leaves thus affected dropped from the plants within 12 days.

3. The distal portions of some of the roots of plants in solution cultures were still capable of some limited elongation after an exposure to 60° for 60 min. The newly formed parts were, however, irregular in diameter and the roots became flaccid and gelatinous after several days. Exposure of roots to 10° for several hours caused them to lose their geotropic sensitivity, resulted in partial inhibition of growth in the apical portions, and produced

an abnormal bending and distortion of such new growth as did arise from the injured apices.

4. It is apparent that cooling of the roots produced wilting through lowering the capacity of those organs to absorb water from without and to transmit it to the conducting channels. Such lowering might be occasioned, to some extent at least, through increase in the viscosity of water, which accompanies cooling; also it might result from a decrease in permeability of root protoplasts or cell walls, due to lowered temperature; or it might result from retardation of some physiological activity of the root cortex (pumping action) induced by cooling. Wilting that occurred while the roots were at an injuriously high temperature may have been occasioned through lowered permeability of the root cortex or through production and upward movement of some sort of toxic material. Slight permanent leaf injury due to cold treatment of roots may perhaps have been due to inadequate water supply while the treatment was in progress, or possibly to toxic material. But the pronounced leaf injury and death of leaf tissues that followed several days after the heat treatment appears to have been due to injurious substances produced in the roots (most of which had been killed by the treatment) or possibly in the adjacent soil. This supposition is supported by the observation that death of heat-treated roots and post-mortem changes therein were accompanied and followed by pronounced discoloration of the stem xylem, which had not been heated.

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# FACTORS IN VARIETAL SUSCEPTIBILITY OF WALNUT FRUITS TO ATTACK BY THE WALNUT-HUSK FLY

A. R. C. HAAS  
(WITH SIX FIGURES)

## Introduction

The varieties of Persian walnuts (*Juglans regia*) differ considerably in the susceptibility of their fruits to attack by the walnut-husk fly (*Rhagoletis completa*). BOYCE (1) has made an intensive investigation of the bionomics of this husk fly and has placed the varieties of walnuts in two groups according to the degree of their susceptibility:

VERY SUSCEPTIBLE	RESISTANT (SLIGHTLY SUSCEPTIBLE)
Eureka	Placentia
Franquette	Seedling
Klondike	Ehrhardt
Payne	Ware
	Neff

The maximum degree of hardness reached by husks during their development (as determined by the puncture-test method) was considered a varietal characteristic. The hardness of the husk was concluded to be the most important factor in determining the susceptibility of a variety to attack by the husk fly. The physical character of the husk in resistant varieties was found to be such that the fly could not puncture the surface layers in order to deposit eggs.

The present studies consider the nature of not only the physical but also the chemical factors involved in the varietal susceptibility of walnut fruits to attack by the husk fly. The results confirm the conclusions of BOYCE (1) regarding the importance of husk hardness and indicate the complexity of the factors involved in such susceptibility.

## Materials

Walnut fruits of three varieties at various stages in their development were secured<sup>1</sup> from an orchard at Elsinore, California. In this location the fruits have not as yet encountered attacks of the husk fly. The varieties of walnut trees were interplanted and the cultural operations in the field were uniform. No dusts or sprays were applied to the fruits at any time.

<sup>1</sup> These fruits were secured through the kindness of Dr. R. S. REID, Oceanside, California.



Walnut fruits of nine varieties were collected on August 24, 1935, from trees growing near Chino,<sup>2</sup> California. These fruits were obtained from trees subject to attacks of the husk fly. Dusts or sprays had been applied to these trees during the season and possibly have had some effect on the husks.

### Methods

Soon after the fruits were picked, they were rapidly washed in running distilled water and were wiped dry. The husks were at once removed<sup>3</sup> by means of stainless-steel knives. The removed husks were transferred to weighed Pyrex dishes (uncovered moisture chambers about 10 in. in diameter and 2 to 3 in. high). Drying was carried on at temperatures below 60° C. in a large well-ventilated oven. When dry the material was finely ground in a Wiley mill.

Husks of samples to be used for pH determinations were placed in rubber-stoppered dry bottles for two days in the freezing compartment of a refrigerator. The juice was then extracted from the husks at 25,000 lb. pressure and the pH values were determined by the quinhydrone electrode.

The number of fruits per sample, the fruit size, variety, fresh and dry weights of the husks, the shells, and the kernels were recorded. In the determination of the ash, the dry plant material was first charred in an electric muffle at low temperature and was then cooled. The salts soluble in hot water were then removed from the char and the residue heated until white. The solution was added to the cooled residues and was evaporated to dryness. After ignition the ash was cooled in a desiccator and then weighed.

Calcium was determined by titration of the oxalate with permanganate; magnesium was weighed as the pyrophosphate; reducing sugars were estimated by the SHAFFER-HARTMANN method, and total pectin was determined as calcium pectate by the method employed by BUSTON (2).

### Experimentation

#### pH IN JUICE OF HUSKS

The pH was determined in the juice extracted from the husks of two collections of the Elsinore fruits. The results shown in table I indicate that the juices in the husks of fruits of a resistant variety such as Placentia are more acid (lower pH) than those in the husks of fruits of susceptible varieties such as Payne and Eureka.

<sup>2</sup> These fruits were collected by Dr. A. M. BOYCE, who was familiar with the varieties grown in that district. Grateful acknowledgment is made for this assistance.

<sup>3</sup> Even though rubber gloves were worn in removing the husks from the shells, the juice of the husks penetrated the gloves and stained the fingers so severely that painful flesh wounds were sustained.

TABLE I  
 DRY MATTER, ASH, AND PH OF WALNUT HUSK JUICE

DRY MATTER IN 100 CC. HUSK JUICE*		ASH IN 100 CC. HUSK JUICE*		PH OF HUSK JUICE*		PH OF HUSK JUICE†	
VARIETY	GRAMS	VARIETY	GRAMS	VARIETY	PH	VARIETY	PH
Neff	7.22	Neff	2.082	Franquette	6.82	July 19, 1935	
Franquette	6.76	Seedling	1.694	Payne	6.01	Placentia	4.70
Placentia	6.53	Payne	1.674	Klondike	5.72	Payne	4.96
Payne	6.33	Ware	1.468	Neff	5.66	Eureka	5.54
Ware	6.09	Klondike	1.447	Ehrhardt	5.65	August 20, 1935	
Seedling	6.07	Ehrhardt	1.359	Eureka	5.54	Placentia	5.20
Eureka	5.92	Franquette	1.276	Placentia	5.46	Payne	5.61
Ehrhardt	5.93	Placentia	1.179	Ware	5.38	Eureka	5.60
Klondike	5.13	Eureka	0.812	Seedling	5.30		

\* Fruits collected August 24, 1935, at Chino, California.

† Fruits collected near Elsinore, California.

The results (table I) for the pH of the juices in the husks of nine varieties of walnut fruits obtained near Chino, indicate that, with one exception (Eureka), the varieties may be placed in the groups proposed by Boyce (1, p. 379) for the susceptibility and resistance of the varieties to husk fly attack in the field. Increased acidity as found in the present studies is therefore associated with greater resistance.

TABLE II  
 DRY MATTER IN HUSKS AND SHELLS (WITH KERNELS) IN FRUITS COLLECTED AUGUST 24,  
 1935, NEAR CHINO, CALIFORNIA

HUSKS		SHELLS WITH KERNELS	
VARIETY	DRY MATTER IN FRESH WEIGHT	VARIETY	DRY MATTER IN FRESH WEIGHT
	%		%
Ehrhardt .....	9.03	Klondike .....	28.60
Seedling .....	10.16	Franquette .....	30.67
Klondike .....	10.21	Eureka .....	33.71
Ware .....	10.22	Ehrhardt .....	36.34
Neff .....	11.12	Payne .....	42.26
Placentia .....	11.14	Neff .....	43.47
Payne .....	11.33	Seedling .....	45.57
Eureka .....	12.25	Ware .....	46.14
Franquette .....	12.29	Placentia .....	46.65

TABLE III  
 DRY MATTER IN HUSKS AND SHELLS (WITH KERNELS) IN FRUITS COLLECTED DURING 1935 NEAR ELSINORE, CALIFORNIA

VARIETY	DRY MATTER IN THE FRESH WEIGHT OF HUSKS					DRY MATTER IN THE FRESH WEIGHT OF SHELLS WITH KERNELS				
	MAY 29	JUNE 7	JUNE 18	JULY 19	AUG. 20	MAY 29	JUNE 7	JUNE 18	JULY 19	AUG. 20
Placentia .....	% 13.16	% .....	% 14.11	% 12.92	% 10.83	% 8.25	% .....	% 10.84	% 21.27	% 39.33
Eureka .....	% .....	% .....	% 14.71	% 14.74	% 11.36	% .....	% .....	% 9.69	% 24.72	% 24.50
Payne .....	% .....	% 13.82	% 14.43	% 13.97	% 10.00	% .....	% 9.53	% 11.61	% 20.79	% 38.65

## DRY MATTER

In table II are given the percentages of dry matter in the fresh weight of the husks of fruits collected near Chino. With the exception of the Klondike, the varieties may be grouped according to the resistance grouping of BOYCE (1, p. 379). The fresh husks of the more resistant varieties have the lower percentages of dry matter and conversely the higher percentages of water. Although the differences are small they are confirmed by the data given in table III for the Elsinore samples. The results (table III) indicate that the fresh husks of the susceptible varieties (Eureka and Payne) generally contain slightly greater percentages of dry matter than those of the resistant variety (Placentia).

When the shells and the contained kernels are considered (table II), the resistance is greater (Ehrhardt variety excepted) with the higher percentages of dry matter in the fresh weight. In the Elsinore samples this was the case only in the August collection.

It is of interest in table III that the percentages of dry matter in the fresh husks reach a maximum in late June and then decrease, while the percentages for the dry matter in the fresh shells (with kernels) increase throughout the season.

Table IV gives data regarding the weights of husks, shells, and kernels on which the computations of the various chemical analyses were based. In the Elsinore samples the dry weights per husk decreased after July 19 although in fruits of the Placentia and Payne varieties the fresh weights were still increasing. Also the dry weights of the shells (with kernels) and of the kernels increased at each fruit sampling although the fresh weights of the shells (with kernels) in Placentia and Payne decreased after July 19. It is of interest that the fresh weights of the husks and of the shells (with kernels) approximate one another at most times during the season and that after June 18 the dry weights of the shells (with kernels) greatly increase over those of the husks. The greater part of this increase occurs in the kernels.

The fresh weights of the husks of the fruits in the Chino samples (table IV) exceeded those of the shells (with kernels) in all varieties with the exception of the Klondike; the dry weights of the husks, however, were considerably less than those of the shells (with kernels).

Table I gives the grams of dry matter in the juice of husks in fruits of nine varieties. The juice was obtained as previously described and was filtered. No relation with the resistance of the husk to the attack of the husk fly was evident.

## ASH, CALCIUM, AND MAGNESIUM

In table I are given the grams of ash in the filtered husk juice in fruits of nine varieties. No relation to husk fly resistance was found. It is seen

TABLE IV  
RELATION OF HUSK TO SHELL AND KERNEL AT VARIOUS STAGES OF DEVELOPMENT

VARIETY	FRUIT SIZE  mm.	DATE OF COLLECTION	No. OF FRUITS USED	AVERAGE WEIGHT PER HUSK		AVERAGE WEIGHT PER SHELL AND KERNEL		AVERAGE WEIGHT PER KERNEL
				FRESH	DRY	FRESH	DRY	
Placentia*	{ 34 × 35 × 40 39 × 36.5 × 44 42 × 39 × 46.5 42 × 39.5 × 46.5	1935 May 29	54	gm. 11.32	gm. 1.49	gm. 9.57	gm. 0.79	gm. 0.77
		June 18	22	17.68	2.50	18.91	2.05	3.94
		July 19	23	21.24	2.74	24.50	5.21	
		August 20	22	21.44	2.32	23.29	9.16	
Eureka*	{ 35 × 33.5 × 46 41.5 × 40 × 52.5 40 × 38 × 50	June 18	47	14.43	2.12	13.32	1.29	0.80
		July 19	25	28.00	4.13	22.45	5.55	2.73
		August 20	22	23.53	2.67	22.65	9.45	
Payne*	{ 32 × 34 × 40 40 × 37 × 50 41 × 38.5 × 45.5 41 × 39.5 × 46	June 7	44	14.85	2.05	10.07	0.96	0.18
		June 18	32	18.45	2.66	23.86	2.77	0.48
		July 19	23	19.91	2.78	19.72	4.10	2.33
		August 20	22	23.18	2.32	19.56	7.56	
Eureka Ehrhardt Seedling Franquette Placentia Payne Ware Klondike Neff		August 24	30	18.37	2.25	17.77	5.99	
		August 24	25	21.18	1.91	17.06	6.20	2.33
		August 24	25	21.66	2.20	16.14	7.36	3.16
		August 24	25	21.90	2.69	17.63	5.41	1.27
		August 24	25	22.30	2.48	17.68	8.25	3.38
		August 24	28	22.38	2.54	19.16	8.10	2.97
		August 24	25	24.78	2.53	22.21	10.25	3.91
		August 24	26	25.50	2.60	33.42	9.67	2.1
		August 24	27	27.57	3.07	24.83	10.79	3.1

\* Fruits collected at Elsinore; the remainder collected at Chino.

from the percentages of ash in the dry matter of the husks (table V) that with the exception of the Placentia variety the high percentages are associated with high resistance to attack by the husk fly. These results are confirmed by the data given in table VI for the fruits collected at various times. With the exception of August 20, on which date the percentage for the Placentia variety is lower than that for the Payne (in agreement with the data in table V), the high percentages of ash are associated with increased resistance.

No association of resistance was found with the percentages of calcium in the dry matter of the husks (tables V, VI).

Except for the Payne variety in the August samplings, the high percentages of magnesium (tables V, VI) were related to increased resistance to husk fly attack. Table VI indicates that, prior to August, the percentages for the Payne and Eureka varieties are less than those for the Placentia.

The changes in the resistance position of the Placentia variety as regards ash and of the Payne as regards magnesium (table VI) is in confirmation of the results of BOYCE, who found that during the latter part of the season the hardness of the husks, which is a factor in resistance, may undergo considerable change.

TABLE V

ASH, CALCIUM, MAGNESIUM, POTASSIUM, SODIUM AND TOTAL PHOSPHORUS AS PERCENTAGES  
IN DRY MATTER OF HUSKS IN FRUITS COLLECTED AUGUST 24, 1935,  
AT CHINO, CALIFORNIA

VARIETY	ASH	VARIETY	CALCIUM	VARIETY	MAGNESIUM
	%		%		%
Seedling .....	15.40	Payne .....	0.82	Payne .....	0.226
Neff .....	14.06	Seedling .....	0.56	Ware .....	0.175
Ware .....	13.39	Placentia .....	0.54	Neff .....	0.125
Ehrhardt .....	13.32	Ehrhardt .....	0.53	Placentia .....	0.123
Klondike .....	13.20	Ware .....	0.52	Ehrhardt .....	0.110
Payne .....	12.00	Eureka .....	0.49	Seedling .....	0.093
Placentia .....	10.63	Neff .....	0.43	Franquette .....	0.087
Franquette .....	9.66	Franquette .....	0.33	Eureka .....	0.073
Eureka .....	8.28	Klondike .....	0.24	Klondike .....	0.064
VARIETY	POTASSIUM	VARIETY	SODIUM	VARIETY	TOTAL PHOS- PHORUS
	%		%		%
Seedling .....	7.71	Seedling .....	0.27	Klondike .....	0.240
Klondike .....	7.04	Ehrhardt .....	0.24	Franquette .....	0.225
Neff .....	6.98	Neff .....	0.17	Neff .....	0.220
Ware .....	6.48	Ware .....	0.14	Ware .....	0.210
Payne .....	5.64	Klondike .....	0.12	Seedling .....	0.193
Placentia .....	5.30	Payne .....	0.10	Ehrhardt .....	0.138
Ehrhardt .....	5.23	Eureka .....	0.10	Eureka .....	0.130
Franquette .....	4.97	Placentia .....	0.09	Payne .....	0.115
Eureka .....	3.91	Franquette .....	0.03	Placentia .....	0.083

The percentages of ash in the husks of the samples from Elsinore (table VI) at first decrease and then increase. The greatest percentage gain in the ash occurred between July 19 and August 20. It is to be noted that, although the ash in the dry matter of the husks (table VI) reaches 12 to 14 per cent., the combined percentages for calcium and magnesium do not reach 1 per cent. HAAS (3) has shown that the dry matter of the husks of Placentia may contain over 10 per cent. of potassium (K), while the dry matter of leaves of this variety may contain less than 1.50 per cent.

#### POTASSIUM, SODIUM, AND TOTAL PHOSPHORUS

Table V shows the high percentages of potassium (K) found in the dry matter of the husks in fruits collected at Chino. With the exception of the Klondike, the varieties that show the greatest resistance to the husk fly attack generally contain the highest percentages of potassium. The Eureka and Franquette varieties are very susceptible and contain the lowest percentages of potassium. The Payne is only slightly out of the order shown in the field, in which this variety would occur between the Ehrhardt and Franquette. The percentages for the Payne, Placentia, and Ehrhardt varieties differ but little from one another.

The percentages for potassium (table VI) show the greatest increase

TABLE VI

ASH, CALCIUM, MAGNESIUM, POTASSIUM, SODIUM, AND TOTAL PHOSPHORUS AS PERCENTAGES IN DRY MATTER OF HUSKS IN FRUITS COLLECTED AT VARIOUS TIMES DURING 1935 AT ELSINORE, CALIFORNIA

IN DRY MATTER	VARIETY	MAY 29	JUNE 7	JUNE 18	JULY 19	AUG. 20
		%	%	%	%	%
Ash	Placentia .....	6.46	.....	5.27	7.12	13.09
	Eureka .....	.....	.....	4.97	6.58	12.40
	Payne .....	.....	5.77	4.76	7.05	13.60
Calcium	Placentia .....	1.02	.....	0.60	0.71	0.54
	Eureka .....	.....	.....	0.79	0.64	0.44
	Payne .....	.....	0.64	0.58	0.44	0.57
Magnesium	Placentia .....	0.214	.....	0.135	0.127	0.056
	Eureka .....	.....	.....	0.114	0.123	0.057
	Payne .....	.....	0.154	0.114	0.119	0.100
Potassium	Placentia .....	2.01	.....	2.16	3.06	6.80
	Eureka .....	.....	.....	1.76	2.79	6.41
	Payne .....	.....	2.32	1.87	3.30	6.71
Sodium	Placentia .....	0.06	.....	0.09	0.11	0.12
	Eureka .....	.....	.....	0.07	0.12	0.08
	Payne .....	.....	0.14	0.12	0.04	0.14
Total phosphorus	Placentia .....	0.175	.....	0.145	0.150	0.200
	Eureka .....	.....	.....	0.133	0.175	0.173
	Payne .....	.....	0.108	0.117	0.147	0.165

during the period from July 19 to August 20. As the results suggest, potassium is the chief basic constituent in the ash of walnut husks.

From the results (table VI) for the Elsinore samples, it is seen that on August 20 the husks of the Placentia and Payne varieties contain greater percentages of potassium in the dry matter than do those of the Eureka, which fact is in agreement with the results shown in table V.

In contrast with the high values found for potassium are the extremely low values found for sodium. In table V, with the exception of the Placentia variety, the more resistant varieties are associated with the higher percentages of sodium.

No relation was evident between resistance and the percentages of total phosphorus (tables V, VI).

TABLE VII  
REDUCING SUGARS AND TOTAL PECTIN (AS CALCIUM PECTATE) IN WALNUT HUSKS  
OF CHINO FRUIT SAMPLES

VARIETY	REDUCING SUGARS IN DRY MATTER OF HUSKS	VARIETY	CALCIUM PECTATE IN DRY MATTER OF HUSKS
	%		%
Neff .....	9.62	Klondike .....	27.31
Payne .....	8.42	Neff .....	20.80
Placentia .....	7.74	Seedling .....	18.11
Ware .....	7.16	Placentia .....	17.79
Seedling .....	6.86	Ware .....	15.29
Franquette .....	6.46	Ehrhardt .....	13.64
Eureka .....	6.21	Franquette .....	11.89
Ehrhardt .....	5.66	Eureka .....	11.01
Klondike .....	2.70	Payne .....	7.33

#### REDUCING SUGARS AND TOTAL PECTIN

The percentages of reducing sugars in the dry matter of the husks of the Chino samples are given in table VII. The Payne and Ehrhardt varieties do not occur in the proper groups according to the resistance of these varieties in the field. Otherwise the high percentages of reducing sugars are associated with high resistance to attack by the husk fly.

In the fruit samples from near Elsinore (table VIII), the dry matter in the husks of the Placentia variety (resistant) in the July and August samples contained higher percentages of reducing sugars than the Eureka and Payne varieties (susceptible). The percentages in the August samples were less than those in the July samples.

In general the higher percentages of reducing sugars are associated with increased resistance. Analyses were also made of the total (as reducing) sugars in order to determine sucrose. The amounts present, however, were too small to permit of an accurate estimate.



TABLE VIII

REDUCING SUGARS AND TOTAL PECTIN (AS CALCIUM PECTATE) IN WALNUT HUSKS OF ELSINORE FRUIT SAMPLES

VARIETY	REDUCING SUGARS IN DRY MATTER OF HUSKS					CALCIUM PECTATE IN DRY MATTER OF HUSKS				
	MAY 29	JUNE 7	JUNE 18	JULY 19	AUG. 20	MAY 29	JUNE 7	JUNE 18	JULY 19	AUG. 20
	%	%	%	%	%	%	%	%	%	%
Placentia .....	7.15	.....	9.49	12.94	7.94	16.04	.....	12.45	13.83	16.09
Eureka .....	.....	.....	11.12	9.74	6.11	.....	.....	15.01	11.62	12.18
Payne .....	.....	10.58	9.76	10.82	5.87	.....	17.19	9.56	12.46	15.91



FIG. 1. Transverse section of husk of the Placentia variety ( $\times 144$ ); wide, compact layer of thick-walled cells close to the epidermis.

The results for total pectin (as calcium pectate) in the dry matter of the husks in the Chino samples are given in table VII. With the exception of the Klondike variety, the high percentages are associated with increased resistance. The data (table VIII) for the July and August samples collected near Elsinore confirm this conclusion.

Pectin is found in the cell walls in leaves (4) and it is assumed to be a cell wall constituent in husks. The greater resistance to attack by the husk fly is associated with high percentages of pectin, and hence the cell wall structure may be a factor in determining husk resistance.

#### CELL STRUCTURE AND ARRANGEMENT OF TISSUES

In addition to the chemical analyses it seemed desirable to make determinations of the physical arrangement of certain tissues that might affect success in the puncturing of the husk by the fly.

The shells of the Payne variety of the Elsinore samples were becoming

hard on June 18, but still could be cut; the shells of the Eureka variety were very soft, while those of the Placentia were hard. The husks in some cases on August 20 could be slipped from the shell.

Sections were prepared of portions of the husks of the Elsinore fruit samples collected on August 20. Figures 1-6 are photographs of transverse sections of these husks. The magnifications for each variety are the same ( $\times 144$  and  $\times 593$ ).

In the husks of the Placentia variety (fig. 1) the tissue that is composed of thick-walled cells is of greater width and compactness than in the husks of the Payne and Eureka varieties (figs. 3, 5). In the husks of the Eureka variety, stone cells are seen at intervals and oriented in a direction perpendicular to the surface.

The sections ( $\times 593$ ) show conspicuous thick cell walls in the husks of the Placentia as compared with those in the husks of the other two varieties.



FIG. 2. Detailed section of Placentia husk tissue extending from epidermis to the heavy-walled cells ( $\times 593$ ). The husk fly encounters difficulty in penetrating this thick-walled cell tissue.



FIG 3 Transverse section of husk of the Payne variety ( $\times 144$ ), relatively few layers of thick walled cells

The results for the cell structure and arrangement of tissues support the conclusion that the husks of fruits of the Placentia variety (resistant) are considerably more difficult for the walnut-husk fly to puncture than are those of fruits of the Eureka and Payne (susceptible)

### Summary

1. A study was made of the complex of factors involved in the varietal susceptibility of walnut husks to attack by the walnut-husk fly. The importance of husk hardness as a factor (Boyce 1) is confirmed, and it is shown that there is a relation between this and other physico-chemical properties of husks in the different varieties.

2. Fruit collections were made at various times from three interplanted varieties of walnut trees located near Elsinore, California. These trees were not dusted or sprayed or subject to husk fly attacks. A single collection of fruits was made on August 24, 1935, from trees of nine varieties

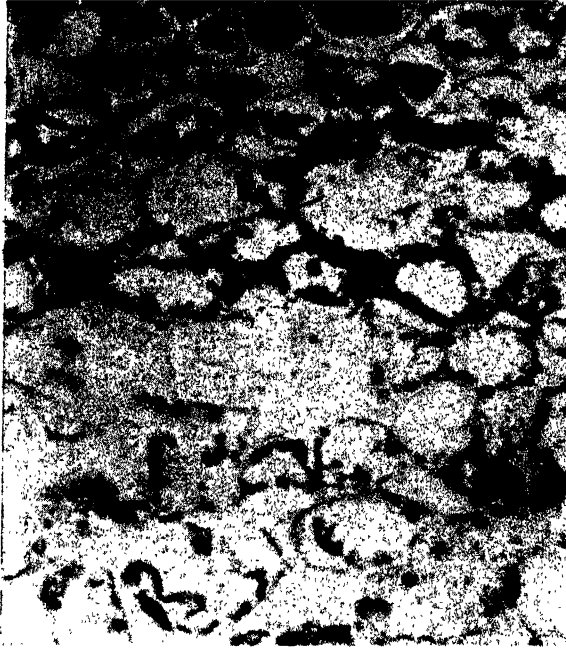


FIG. 4. Detailed section of Payne husk tissue extending from the epidermis ( $\times 593$ ), showing few, if any, thick-walled cells.

grown near Chino, California. These trees were subjected to dusts or sprays and to attacks of the husk fly.

3. With one exception (Eureka variety among the Chino samples) the pH of the juices of the husk appears to be related to the resistance to husk fly attack in the field. Increased acidity is associated with greater resistance.

4. Increased resistance is generally accompanied by a smaller percentage of dry matter in the fresh husks. In the August samples the resistance usually was associated with high percentage of dry matter in the fresh weight of the shells (with kernels).

5. The dry weights of the husks decreased after July 19 even though in fruits of the Placentia and Payne varieties the fresh weights were still increasing. The dry weights of the shells (with kernels) and of the kernels increased at each fruit sampling. The fresh weights of some varieties of shells (with kernels) decreased after July 19. The fresh weights of husks generally exceeded, while the dry weights were much less than the corresponding weights of the shells (with kernels).

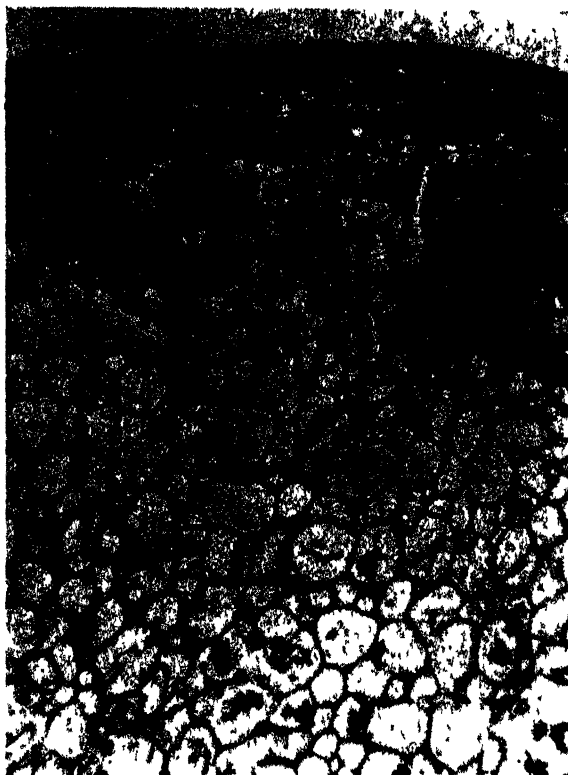


FIG. 5. Transverse section of husk of the Eureka variety ( $\times 144$ ); scattered, small thick-walled cells interspersed with clusters of heavy-walled cells oriented perpendicular to the epidermis.

6. No relation with the resistance to the attack of the husk fly was found in the grams of dry matter or in the grams of ask in the husk juices.

7. High percentages of ash, magnesium, potassium, sodium, reducing sugars, and total pectin in the dry matter of the husks were associated with increased resistance, while no relation of resistance to calcium or total phosphorus in the dry matter was evident.

8. Studies of transverse sections of the husks of Placentia, Eureka, and Payne varieties indicate the importance of cell-wall thickness and the distribution of these tissues. Husk hardness associated with resistance is largely due to the cell-wall structure and to the distribution of the husk tissues. Husks of fruits of the Placentia variety (resistant) possess thick-walled cell tissues of greater width and compactness than those of fruits of the Payne and Eureka varieties (susceptible).

9. Very little data exists in regard to the composition of walnut kernels,

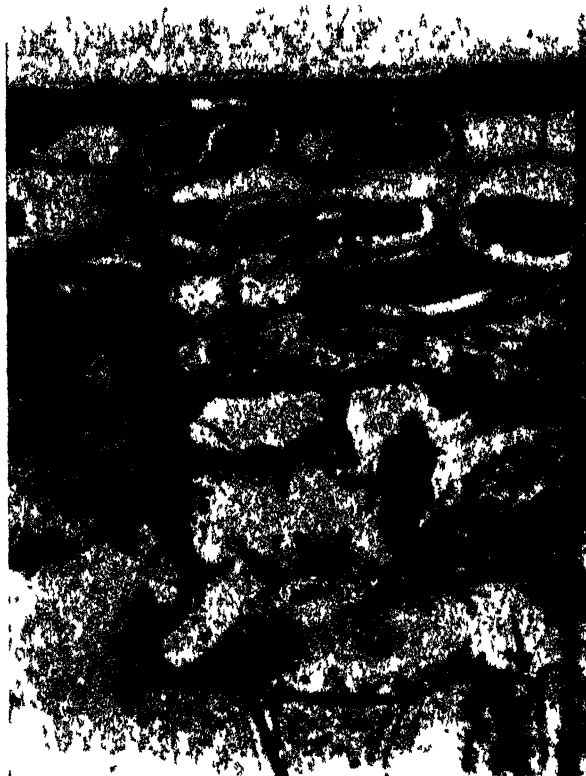


FIG. 6. Detailed section of Eureka husk tissue showing very few thick-walled cells ( $\times 593$ ).

shells, and husks. The present studies afford extensive data in regard to the composition of walnut husks.

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# POLAR TRANSPORT OF AUXIN AND ELECTRICAL POLARITY IN COLEOPTILE OF *AVENA*

W. G. CLARK

(WITH ONE FIGURE)

## Introduction

In the introduction to a recent paper by the writer (5), the phenomenon of the polar transport of auxin in plants was briefly discussed. It was indicated there that the electrical theory of the polar transport of auxin was one of the most widely accepted ones. This theory states that the inherent electrical polarity of plants is the cause of the longitudinal polar transport of the negative ion of auxin, the plant-growth hormone, to an inherent positive pole in the plant.

It was concluded (5) that an electrical polarity exists in seedlings. Apices are electronegative to basal regions in seedlings of *Impatiens*, *Avena*, *Zea*, *Pisum*, and *Vicia*. In *Avena*, this polarity exists internally as well as on the surface of the cuticle. It is thus seen that the electrical polarity found in seedlings conforms with that demanded by the electrical theory of the polar transport of auxin.

Cut sections of these seedlings exhibited the same electrical polarity as intact plants, i.e., electronegativity of the morphological apical cut surface. But it was also shown that this section-polarity could be inverted by gravity; and Clark (4) showed that the electrical polarity of the *Avena* coleoptile could be altered or inverted by light. Thus the electrical theory of polar transport is directly contradicted by the fact that neither light nor gravity affect the longitudinal transport of auxin (See "Discussion" and 5).

The present article is concerned with experiments in which attempts were made to link polar transport and electrical polarity. Auxin transport and electrical polarity were measured at the same time, and then the electrical polarity was altered by applied potentials and by gravity to see if there were any parallel changes in polar transport.

## Experimentation

### EFFECT OF APPLIED P.D.'S ON AUXIN DIFFUSION IN AGAR

In accordance with an electrical theory of transport of the plant-growth hormones, it was of primary importance first to demonstrate whether or not the hormone is electrically transported *in vitro*.

DOLK (6) attempted to demonstrate such an *in vitro* transport of auxin by the following method: *Zea Mays* growth substance (auxin-A) was collected by diffusion from coleoptile tips into agar blocks. Two such blocks



were interposed between platinum electrodes, and a P.D. of from 2 to 10 volts (v.) applied across them of one hour. At 10 v., a strong electroosmosis of water occurred, causing one block to take up water and swell, and causing the other block to lose water and shrink. DOLK stated that such electroosmosis would distort the results (the water current would carry the auxin to the negative pole). He also said that electrolytic destruction of auxin would probably occur at the electrodes. At 2 v., no appreciable electroosmosis occurred, but the auxin concentration in the two blocks remained the same, as tested by the standard *Avena* test (see text below). At 10 v., no auxin could be found owing to its destruction. DOLK concluded that auxin was not electrically transported under the conditions of his experiments.

KOCH (12) also investigated this problem. He placed 15 to 18 *Avena* coleoptile tips on agar blocks for 90 to 120 minutes to collect their auxin by diffusion, while passing a current from a 4-v. flashlight battery for 1 to 2 hours through the agar block between two electrodes (platinum wire). The block was then removed, cut into small blocks, and tested on *Avena*, using 2 plants per test. He claimed that the auxin was transported to the positive pole, which is theoretically in the direction expected of a dissociated acid. His experiments are to be severely criticized in that he used too few test plants. Usually 12 test plants give an average result correct within 10 per cent (see text below). Individual differences between two plants may be considerable.

The writer has reinvestigated the problem of electrical transport of auxin. Synthetic heteroauxin (indole-3-acetic acid) prepared in the Gates chemistry laboratories was used. Since this substance is a weak acid, it dissociates into a large anion and a hydrogen ion. The anion should be transported in an electric field to the anode. That heteroauxin conducts a current can be determined by actual conductance measurements.

In order to demonstrate that the heteroauxin used in the experiments about to be described actually conducts a current, conductance measurements were made so that the transport number and absolute mobility under unit potential gradient could be ascertained. The sodium salt of indole-3-acetic acid was prepared by accurately neutralizing the acid and recrystallizing from absolute alcohol. (The salt preparation was made by Dr. KOEFLI of the California Institute of Technology.) The salt was then made up in different concentrations and their conductances determined in a calibrated conductance chamber at 25° C., using a conductance bridge with a Vreeland oscillator at 1000 cycles. The cube root of the concentrations was plotted against the equivalent conductances. This curve was extrapolated to the limiting conductance at infinite dilution. Since the limiting conductances of the hydrogen and of the sodium ions at 25° C. are known, the limiting

conductance of the acid could be determined from that of the experimentally determined conductance of the more dissociated salt. Knowing the limiting conductance of the acid, the dissociation constant could be calculated in order to check the accuracy of the conductance measurements. The dissociation constant is known to be about  $2.0 \times 10^{-5}$  at  $25^\circ \text{C}$ . (15, 7). From the limiting conductance of the heteroauxin anion, its transport number and ionic mobility could be calculated.

Two experiments gave limiting conductances of the salt of 79.5 and 78.5, average 79.0.

Since  $\Lambda_0 \text{ Na at } 25^\circ \text{C.} = 50.9$  (NOYES and SHERRILL, 17),  
 and  $\Lambda_0 \text{ H at } 25^\circ \text{C.} = 350$  (*ibid.*);  
 hence  $\Lambda_0 \text{ auxin anion} = 79.0 - 50.9 = 28.1$ ,  
 and  $\Lambda_0 \text{ auxin} = 28.1 + 350 = 378$  at  $25^\circ \text{C}$ .

Since the dissociation constant,  $K$ , is proportional to the degree of dissociation; hence

$$K = \frac{C a^2}{1 - a}, \text{ where } a = \frac{\Lambda}{\Lambda_0}, \Lambda =$$

the conductance at different concentrations, and  $C$  = the concentration in mols. For three concentrations used, the dissociation constants were:

Normality	$K \times 10^{-5}$
0.004	3.0
0.008	2.8
0.01	2.8
<hr/>	
Av. 2.9	

For a comparison, KÖGL and KOSTERMAN's values for the dissociation at  $25^\circ \text{C}$ ., their determinations being based on pH measurements, are given:

Normality	$K \times 10^{-5}$
0.001	2
0.005	3
0.01	1
<hr/>	
Av. 2	

DOLK and THIMANN (7) determined the dissociation constant of heteroauxin from *Rhizopus suinus* by partition coefficients and the dissociation at various pH's. Their average value (at room temperature) was 1.82, hence approximately that of KÖGL and KOSTERMANS.

It is seen therefore that the conductance measurements were quite accurate, and in fact gave better constants than either KÖGL and KOSTERMANS' or DOLK and THIMANN's.

The transport number of the heteroauxin anion is equal to the ratio of the limiting conductances of the anion to that of the sum of the anion and cation:

$$28.1/378.1 = 0.07$$

Hence only 0.07 of an electric current is carried by the heteroauxin anion in solution. The absolute mobility of the anion under unit potential gradient of 1 v. per centimeter in a cube 1 cm. on each side is:

$$\begin{aligned} 28.1/96,500 &= 2.9 \times 10^{-4} \text{ cm./sec.} \\ &= 10.5 \text{ mm./hr.} \end{aligned}$$

Before experimenting with applied P.D.'s in plants, it was of interest to see how much of a potential gradient is necessary to establish a *measurable* difference in transport in a liquid or agar medium. The transport of heteroauxin to the anode can be obtained if a sufficiently high P.D. is used. The following experiments demonstrate that the electrolytic migration might easily escape observation unless the proper precautions are taken.

The following experiment shows a typical negative result:

Tap-water agar blocks  $11 \times 8 \times 1$  mm. were soaked one hour in heteroauxin of a known concentration. One hour of soaking permitted complete equilibrium between block and solution. One of the blocks was then placed atop three tap-water blocks containing no auxin, and Ag-AgCl electrodes (freshly plated) applied at each end of the stack of blocks. A P.D. of 300 mv. (millivolts) was applied for one hour such that a 9 microampere current flowed. Another stack of blocks was set up in the same way, but with the polarity of the applied P.D. reversed. A control stack was run in which no current was passed. At the end of the hour, the blocks were removed, separated, and each one cut into 12 smaller blocks and tested for their auxin content by the standard *Avena* test.<sup>1</sup>

Five experiments of the kind just described were performed, and table I records typical results.

In another experiment, a P.D. of 450 mv. applied for one hour, at 28 microamp. of current-flow gave considerable electrolytic destruction of auxin. The concentration used in the top blocks was 20°. At the end of one hour only about 5° remained in all four blocks. The blocks nearest the anode

<sup>1</sup> The technique involves placing a small agar block containing auxin on one side of the stump of a decapitated *Avena* coleoptile so that the auxin from the block will diffuse down only along the side under the agar block, thereby causing this side to grow more than the other side. This will result in a curvature away from the side on which the block was placed. The curvature is proportional to the concentration of auxin in the block, to the size of the block, to the time between decapitation and placing the block, and to the time that the block is left on. The method is so controlled (temperature, humidity, light, tools, time, plants, etc.) that within certain limits of auxin concentration, the curvatures in degrees give a quantitative analysis of the concentration such that 1° curvature per 10-mm.<sup>3</sup> block corresponds to about  $10^{-7}$  to  $10^{-9}$  mg. of heteroauxin. (This again depends upon the methods used and the sensitivity of the plants.) Usually 12 to 24 test plants are used for each determination, the averages being taken. The accuracy is about 10 per cent. when 12 plants are used. For detailed descriptions of the method, and other test methods, see WENT and THIMANN (28).

TABLE I

EFFECTS OF APPLIED P.D. ON AUXIN DIFFUSION OF AGAR AT LOW VOLTAGE  
P.D., 300 mv.; CURRENT-FLOW, \* 9 MICROAMP.; TRANSPORT TIME, 1 HR.

BLOCKS	DEGREES OF CURVATURE (AUXIN CONCENTRATION)		
	Control	Anode	Cathode
Top .....	12	9	10
	6	7	5
	5	2	4
Bottom .....	2	2	2
		Cathode	Anode
Total (sum) .....	25	20	21

\* The arrows indicate the theoretical effect of the current flow on the auxin transport.

showed white discoloration which, on microscopic examination, proved to be due to minute gas bubbles, presumably of oxygen. When 10° auxin was used, and 600 mv. applied, complete destruction occurred.

The above experiments show clearly that when no electroosmosis or electrolytic destruction occurred, the applied P.D. had no effect on the transport of auxin in agar. Evidently higher voltages are necessary to effect a measurable change in transport. But at higher voltages, destruction by electrolysis occurs. Therefore it is easy to understand why DOLK obtained negative results.

Transport experiments in agar were then conducted, in which much higher voltages were used. Automatic siphons were used to wash away the products of electrolysis at the electrodes so that no auxin destruction might occur. Zinc electrodes were used, and the solutions at the electrodes were separated from the agar blocks by agar-gelatin seals. A 25° auxin solution was used to wash the top electrode. Analysis of the four blocks used in the experiment is given in table II.

TABLE II

P.D., 22.5 v.; CURRENT-FLOW, 0.1 MA. (MILLIAMPERE); TRANSPORT TIME, 1 HR.  
EFFECTS OF APPLIED P.D. ON AUXIN DIFFUSION AT HIGH VOLTAGE

BLOCKS	DEGREES OF CURVATURE (AUXIN CONCENTRATION)		
	Control	Cathode	Anode
Top .....	6	26	0
	3	24	0
	2	19	0
	1	12	0
Bottom .....		Anode	Cathode

The control shows that transport by diffusion was much less than electrolytic transport. This P.D. represented approximately a 50-volt-per-centimeter gradient, and definitely causes auxin to be electrolytically transported to the positive pole. The results are not qualitatively distorted by electro-osmotic-flow, since water travels to the negative pole in agar and would only tend to decrease the amount of auxin carried by electrolysis to the positive pole.<sup>2</sup>

#### ELECTRICAL POLARITY AND POLAR TRANSPORT IN *AVEN*4 COLEOPTILE

It has been demonstrated that sections of coleoptiles exhibit an electrical polarity which is in the direction expected by WENT's theory of electrical transport (*cf.*, 5), *i.e.*, the morphological apices are electronegative to the bases, and the negatively charged ion of the acid, auxin, could, then, theoretically be electrically transported polarly from tip to base. The following section is concerned with experiments attempting to directly demonstrate that polarity of auxin transport varies in the same way that electrical polarity varies when it is altered.

#### TRANSPORT TECHNIQUE

The transport experiments were performed as described by VAN DER WEIJ (25), except that no holders were used to support the coleoptile sections, as this causes auxin leakage through water films in the holder. The seedlings were grown in sand from hulled seeds of *Avena sativa* (Victory oats) of a pure line stock (also used in the *Avena* tests). Germination, growth, transport experiments, and tests were all carried out in the experimental dark room at a relative humidity of 90 per cent., at 24° C., and in orange light not causing phototropism. The lengths of the sections used were in all cases 3 mm. They were cut from the subapical zones of coleoptiles 3 to 4 cm. in length, one to two sections per coleoptile. The section-cutters were made of two parallel razor blades separated by a brass strip. The sections were then placed in an upright position on wet filter paper by means of eye-forceps. This washes out enzymes which destroy auxin (18). After an hour of such "washing," the sections were removed, and excess moisture carefully removed from the sections with filter paper. Twenty sections were each then placed upright on the 11 × 8 × 1-mm. agar blocks (tap-water agar) and another block containing the auxin placed on top of the sections. The sections were handled with fine eye-forceps. Agar blocks can be made to contain auxin in any desired concentration by soaking them in auxin solutions of known concentration for about an hour. The

<sup>2</sup> That water is carried to the negative pole was easily demonstrated by applying a P.D. of 1 or 2 v. across an agar thread 20 × 1 mm. The thread immediately swelled at the negative pole, and shrunk at the positive pole. Therefore, in the above experiment, auxin traveled to the positive pole *despite* any flow of water in the opposite direction.

transport time was usually one to two hours, depending upon the concentration of auxin in the top block. After this time, the two blocks were removed, cut into 12 smaller blocks, and tested on *Avena*. The sum of the values obtained for each block should approximate the original amount in the top block. The amounts of auxin transported were expressed as the percentages of the sums of the concentrations in top and bottom blocks present in the bottom blocks.

SHUNTING EXPERIMENTS.—ROSENE (22) demonstrated that the P.D. between apex and base of an *Allium cepa* root could be reversibly decreased by liquid shunts interposed between the contacts, and at a distance from them. On the basis that the P.D. between the two ends of a coleoptile section could be lowered by a shunt, it was thought that auxin transport might be simultaneously lowered. Hence metallic shunts (metal foil) were made between tops and bottoms of such sections, and transport experiments run. In the set-up of the experiment clean tin-foil connected the top agar block with the bottom agar block. Tin-foil not connecting the blocks was applied to the blocks of the controls. The results of a typical experiment are given in table III.

TABLE III

EFFECTS OF SHUNTING THE P.D. ON TRANSPORT OF AUXIN

TRANSPORT TIME, 1 HR.; LENGTH OF SECTIONS, 3 MM.; 20 SECTIONS PER EXPERIMENT

BLOCKS	DEGREE OF CURVATURE (AUXIN CONCENTRATION)	
	Shunted	Control
Top	8	8
Bottom	3	4.5
Total (sum.)	11	12.5
	Percentage of total curvature (sum)	
Bottom	% 27	% 36

The difference between the shunted and the control experiments, -9 per cent., is well within the experimental error.

In five similar experiments the differences in percentage of curvature (expressed as the above result, -9 per cent.) were 12, 9, -7, -5, and -2 per cent., with an average of 1.4 per cent., which is within the experimental error.

The results of the experiments showed that shunting the P.D. between the two ends of a section has no effect on the auxin transport. It is realized that such a shunt is probably very ineffective in reducing the individual

P.D.'s probably maintained across each cell. The electrical resistance of the coleoptile section is very high ( $10^5$  ohms for 20 3-mm. sections in parallel), and the P.D. between the two ends is relatively very minute (a few millivolts). Hence any current dissipated by metallic shunts between the two cut ends would be extremely small, and the individual cell P.D.'s would be relatively unaffected. If the resistance of the tissue had been quite small, and there were relatively few cells between the electrodes, the situation might have been quite different (8). In the work of FRANCIS (8) the P.D. across a frog's skin was shunted through a total resistance of 1500 ohms, and the current-flow was measured. If the external resistance had been lower, the transport of ions across the skin might conceivably have been altered.

#### APPLIED P.D.'S AND TRANSPORT IN COLEOPTILE SECTIONS

KÖGL (13, 14) claimed to have found an effect of applied potentials on longitudinal transport in *Avena* coleoptiles, produced by placing agar blocks containing auxin on one side of decapitated coleoptiles and applying a P.D. between this block and the base of the plant. The curvatures resulting were increased when the tip was made the cathode, and decreased over the controls when it was made the anode. The auxin anion was presumably transported in the plant toward the anode, and prevented from being transported downward when the base was made the cathode. P.D.'s of 0.2 to 1.4 v., and currents of 0.4 to 2.8 microamp. were used. Later, however, KÖGL, HAAGEN-SMIT, and VAN HULSEN (16), stated that the applied currents merely caused the auxin to be transported more rapidly from the agar into the plant, and that the effect was not on the longitudinal transport in the plant.

The effect of electric fields on growth of plants has been reviewed by WENT (27), RAMSHORN (20), and STERN (24). Even if some of the results could have been explained by the effects of electric currents on the transport of auxin in the plants, the results are conflicting, to the extent that no agreement has been reached that direct currents of one polarity affect growth in a definite way.<sup>3</sup>

Since it had not yet been shown that auxin will be longitudinally transported in plants under the influence of an electric current, it was important to obtain experimental evidence to this effect.

Transport experiments, such as described in the previous section, were performed while a P.D. was applied between the two blocks in such a way that the electrical current would theoretically augment transport in one case, and inhibit it in the other. Ag:AgCl electrodes were applied directly to the agar blocks, and P.D.'s of 25 to 750 mv. applied for 1 to 2 hours. The

<sup>3</sup> A paper by CHOLODNY and SANKIEWITSCH in the April no. 1937, *PLANT PHYSIOLOGY*, describes the use of applied currents which were found to increase coleoptile growth if the apex is made the cathode, and to inhibit it if the apex is made the anode. Immediately after cutting off the current, however, inhibition ensued.

electrodes were the same size as the agar blocks, and were replated before every experiment. In a typical experiment heteroauxin concentration was  $40^\circ$  in the top block at the start. After one hour transport time, the top blocks were diluted 3X for analysis. The results of this experiment are seen in table IV.

TABLE IV

EFFECTS OF APPLIED P.D.'s AND TRANSPORT OF AUXIN IN COLEOPTILE SECTIONS  
P.D., 300 mv.; TRANSPORT AND CURRENT-FLOW, 1 HR.; 20 3-MM. SECTIONS PER EXPERIMENT

BLOCKS	DEGREES OF CURVATURE (AUXIN CONCENTRATION)		
	Control	Anode	Cathode
Top .....	35	31	31
Bottom .....	8	7 ↑	7 ↓
		Cathode	Anode
Total (sum.) .....	43	38	38
	Percentage of total curvature (sum)		
	%	%	%
Bottom .....	18.6	18.4	18.4

In twelve experiments like the preceding in which the applied P.D. varied from 25 to 300 mv., the total average percentage of the sum of auxin concentration expressed as percentage of total curvature in the bottom block is shown in table V.

TABLE V

EFFECTS OF APPLIED P.D.'s AND TRANSPORT OF AUXINS IN COLEOPTILE SECTIONS

BLOCKS	PERCENTAGE OF TOTAL CURVATURE (SUM) (AUXIN CONCENTRATION)		
	Control	Anode	Cathode
Top .....	%	%	%
	25	21 ↑	20 ↓
Bottom .....		Cathode	Anode

It is obvious that no effects of applied P.D.'s on transport were found.

Since no results were obtained in the above experiments, it was thought that perhaps the potential gradients were not steep enough. The absolute mobility of the heteroauxin anion is 10.5 mm. per hour at 1 v. per centimeter. We should, therefore, expect 300 mv. per 3 mm., which is nearly 1 v. per centimeter, to suffice, regardless of the fact that the conducting path in the experiments just described is not exactly like the standard cube usually



referred to when the term, "absolute mobility," is used. It was found, however, in the experiments using Ag, AgCl electrodes, that a potential difference of more than 300 mv. caused destruction of auxin. It must be remembered that hydrogen ions, and ions of other salts to be found in agar and tap water, will carry the current, the auxin carrying only 7 per cent. of the current. Hence it would be necessary to apply larger currents for longer periods of time, especially when high resistances are encountered (e.g., 50 v. per cm. were necessary in the case of agar).

Since electrolytic destruction of auxin occurred when P.D.'s above 300 mv. were used, recourse to flowing electrodes was taken. The same type of siphon-flow electrode vessels were used as were described in the section on transport in agar, except that the apparatus was made so that the electrode vessel and top-agar block could be racked down to the top of the sections placed on the bottom block, by means of a set-screw mechanism. This dispensed with clumsy manipulation and the danger of upsetting the sections, or of injuring them by loading them down with excess weight. The fluid used for washing the top electrode was 0.1 N KCl with auxin, and for washing the bottom electrode 0.1 N KCl without auxin. Current was passed so as to theoretically augment transport in one case, and to decrease it in the other (opposite polarity). No difference was found, however, in the amounts of auxin transported, even when P.D.'s of 3 v., and a current of 0.04 ma. was used. The sections, after current treatment, were "washed" in an upright position on wet filter paper, and tested in further transport experiments to see if there were any after-effects of the current treatment, but none were observed. It was not easy to wash out the auxin remaining in the sections left there by the first treatment, so that any after-effects occurring might be masked by contamination. Hence sections were current-treated in the apparatus *without* auxin application, and then immediately tested in transport experiments for any changes in their ability to transport normally. A description of part of the set-up of a typical experiment to determine the after-effect of applied P.D. on transport follows. Forty 3-mm. sections from subapical zones of coleoptiles were used for each experiment, i.e., for each polarity of the applied P.D., and for the control. Each lot of 40 sections in each experiment was then halved for duplicate transport experiments, using an auxin concentration of  $15^\circ$  in the top blocks. The average results of each duplicate set are shown in table VI.

The results of the average of four experiments like the preceding are given in table VII as the percentage of the total amount of auxin in top and bottom present in the bottom blocks. These results signify no effect of the current on transport in the sections. Higher currents, i.e., above 1 ma., caused the sections to become flaccid, and hence could not be used in any of the transport experiments, as was found necessary in the case of agar.

TABLE VI

AFTER-EFFECTS OF APPLIED P.D. ON TRANSPORT OF AUXIN

P.D., 1.5 v.; CURRENT-FLOW, 0.8 MA.; CURRENT TREATMENT 1 HR.; TRANSPORT-TIME 1 HR.

BLOCKS	DEGREES OF CURVATURE		
	Control	Anode	Cathode
Top .....	7.2	7	7
Bottom .....	6	5 ↑	4.6 ↓
		Cathode	Anode
Total (sum.) .....	13.2	12	11.6
	Percentage of total curvature (sum)		
	%	%	%
Bottom .....	45	42	40

TABLE VII

PERCENTAGE OF AUXIN IN BOTTOM BLOCKS

BLOCKS	PERCENTAGE OF TOTAL CURVATURE (SUM)		
	Control	Anode	Cathode
Top .....	%	%	%
	38	37 ↑	39 ↓
Bottom .....	.....	Cathode	Anode

#### EFFECT OF APPLIED P.D.'s ON INHERENT P.D.'s AND TRANSPORT IN *AVENA* COLEOPTILE SECTIONS

The question now arose: Does the inherent P.D. of the sections change appreciably when such P.D. applications are made? To answer this question, transport experiments were performed in which the transport, inherent and applied P.D.'s, were measured simultaneously.

Several such experiments were performed, and the following was a typical setup. Three-mm. sections were cut and washed in an upright position for 1 hr. on wet filter paper. Twenty such sections were placed on tap-water agar blocks in a moist chamber, electrical contact being made to the block with a strip of 0.1 N KCl agar which led through paraffined glass tubes outside the moist chamber to a cup filled with 0.1 N KCl in which the Zn: ZnSO<sub>4</sub> electrode could be placed. The top agar block containing auxin had a similar KCl-agar strip leading to another cup and electrode outside the chamber. The control transport experiment had KCl-agar strips of the same size contacting the top and bottom blocks. The inherent P.D.'s of such a setup were measured with the string electrometer, as well as the applied P.D.'s. The current was measured with a microammeter. It was found

that the electrodes did not polarize with the currents used, so that the same electrodes used for applying the P.D.'s could be used for measuring the change in inherent P.D.'s of the sections. In such a transport experiment, it is seen that the P.D.'s of 20 sections in parallel are measured, thus the average. P.D.'s were measured and applied, cut off after a time, and the inherent P.D.'s immediately measured. About 2 seconds elapsed between two such readings, but it was observed that the inherent P.D.'s maintained their levels several seconds after current treatment of a minute or so. In other words, depolarization was not so rapid that the effect of the applied P.D.'s on the inherent P.D.'s could not be accurately determined with the short-period string electrometer.

The transport time was 90 minutes; the applied P.D., 3 v.; and the current-flow, 10 microamp. *Before* the P.D. was applied, the inherent P.D.'s were: Cathode at the top (current flow theoretically augmenting transport), apex 2 mv. negative to the base; anode at the top (current theoretically inhibiting transport), apex 4 mv. negative to the base. Hence the same polarity existed as was found in an earlier part of this paper. 5 minutes after the current was turned on, the inherent P.D. was measured, after briefly cutting off the applied current. Every 5 minutes thereafter during the course of the experiment, the inherent P.D. was measured and recorded, and found to be the same throughout, namely: Cathode at the top (applied polarity same as inherent), apex 43 mv. negative to base; anode at the top (applied polarity opposite inherent polarity), 55 mv. *positive* to base. The blocks were analyzed for their auxin content at the end of the 90 minutes. The results obtained are given in tables VIII and IX.

TABLE VIII

EFFECT OF APPLIED P.D.'s ON INHERENT P.D.'s AND TRANSPORT IN *AVENA*  
COLEOPTILE SECTIONS  
INHERENT APICAL NEGATIVITY INCREASED FROM 2 TO 43 MV.

BLOCKS	DEGREE OF CURVATURE (AUXIN CONCENTRATION)	
	Control	Cathode
Top .....	4	3
Bottom .....	5	4
		Anode
Total (sum.) .....	9	7
	Percentage of total curvature (sum)	
	%	%
Bottom .....	56	57

TABLE IX

EFFECT OF APPLIED P.D.'S ON INHERENT P.D.'S AND TRANSPORT IN *AVENA*  
COLEOPTILE SECTIONS

INHERENT POLARITY INVERTED FROM APICAL NEGATIVITY OF 4 MV. TO A POSITIVITY OF 55 MV.

BLOCKS	DEGREE OF CURVATURE (AUXIN CONCENTRATION)	
	Control	Anode
Top .....	4	4
Bottom .....	3	3 ↑
	.....	Cathode
Total (sum.) ....	7	7
	Percentage of total curvature (sum)	
Bottom .....	43	43

Five experiments like the preceding gave the same result, namely, that inverting or increasing the inherent electrical polarity had no effect on the polar transport.

## GEOELECTRICAL POLARITY AND POLAR TRANSPORT OF AUXIN

WENT (26), VAN DER WEIJ (25), and PFAELTZER (19) have clearly demonstrated that gravity has no effect on the polar transport of auxin in *Avena* coleoptile sections. It has been shown in a recent paper by the writer (5) that inverting sections inverts their electrical polarity for a time. Should it be shown that this inverted polarity, during the time that it exists, has no effect on polar transport, the evidence would be stronger against the thesis of electrical transport. To this end, transport experiments were performed, in which sections were inverted, and the inherent electrical polarity and transport of auxin were measured. Figure 1 gives the results of such an experiment. The setup of this experiment was as follows:

Sections were cut and divided into two lots. One lot was placed on wet filter paper in an upright position; the other lot was placed in an inverted position. After 1 hour, 20 sections from each lot were removed and a 90-minute transport experiment run on each lot, the sections remaining in the position in which they had been placed on the filter paper. One auxin agar block was placed on the inverted apices of the inverted lot, and another on the upright apices of the upright lot. During the transport, the P.D. between each block of the two transport experiments was measured several times during the experiment. The curves in figure 1 represent the change in these P.D.'s with time. At the end of two hours, two more lots were removed from the wet filter paper and the process repeated, as was also done for two more lots left standing 4 hours after sectioning. In this way, the

effect of gravity could be followed over a period of several hours. Figure 1 contains the results for the upright and inverted sections left in those respective positions in transport experiments for 1, 2, and 4 hours, thus including six experiments. The P.D.-time curves of each of the six experiments; and diagrams representing the auxin analyses of the top and bottom blocks, together with the tabulated sums of the top and bottom blocks, and the percentage of this sum in the bottom blocks, are also shown in figure 1.

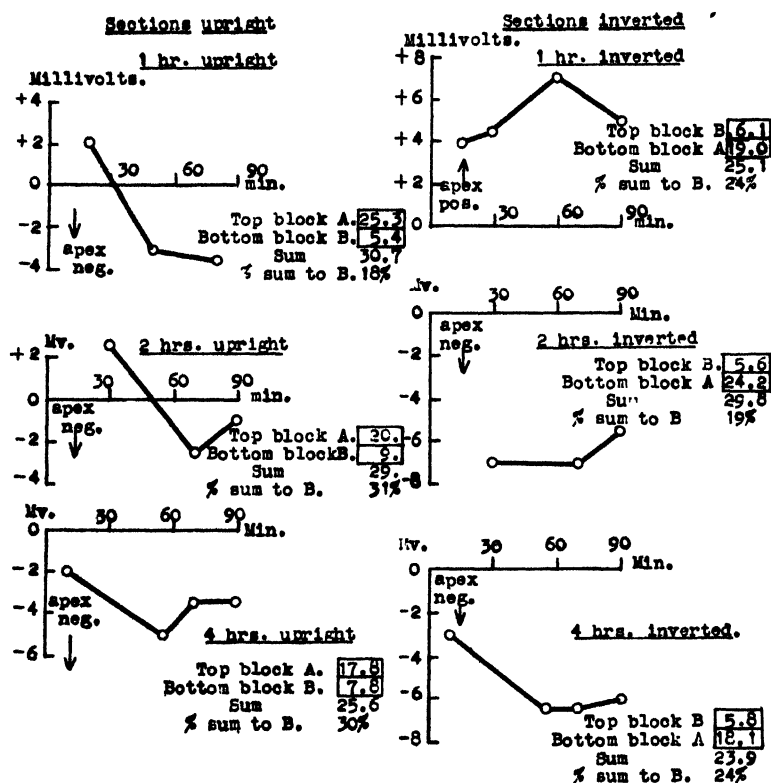


FIG. 1. Effect of inverting on electrical polarity and auxin transport.

EFFECT OF INVERTING ON ELECTRICAL POLARITY AND AUXIN TRANSPORT.—In the case of the upright sections, the P.D.-time curve shows the usual characteristics, namely, that time is necessary before the apical negativity is established, in this case less than an hour (5); and, in the case of the sections left standing 4 hours, this polarity was already established. It is seen that the sections inverted one hour show the usual inverted electrical polarity, *i.e.*, apical positivity, and that this positivity is maintained throughout the transport experiment; hence if the electrical transport theory were correct, the *transport* polarity should also be inverted. After two hours of

inversion, the inverted sections have regained their normal polarity, a characteristic already discussed in the foregoing pages. After 4 hours, the normal polarity was even more pronounced.

It is seen at once that regardless of the fact that the electrical polarity was inverted, in the case of the sections left inverted one hour, the amount of auxin transported is the same as in the upright section with normal polarity. In all cases in figure 1, the differences (in percentages of the sums of top and bottom blocks found in the bottom blocks) between transport experiments on upright and inverted sections were within the limits of error.

The results reported here clearly demonstrate a close parallelism between the known direction of auxin transport inside the plant and the *normally* existing electrical polarity described in the writer's recent paper (5), namely: that the tip of the coleoptile of *Avena* is electronegative to the base; and the dissociated anion of heteroauxin could be electrolytically polarly transported to the electropositive base. That auxin is polarly transported has also been shown by evidence discussed in the introduction to the writer's recent paper (5). It was then shown that this electrical polarity could be changed and inverted by means of applied electromotive forces, and by gravity (and also by light, as was shown in an earlier paper, (4)); while the amount and polarity of auxin transport remains the same. These facts were taken to mean that the polarity of auxin transport and electrical polarity are independent of one another.

The works of BRAUNER and BÜNNING (3), KOCH (12), and AMLONG (1) have shown that the lateral transport of auxin in plant organs is probably linked with geo- and photoelectric P.D.'s, or at least have shown a close relationship between the two (5). These P.D.'s were measured in approximately the same way as described above. Moreover, light, gravity, and applied P.D.'s can cause tropisms. Light and gravity induce lateral transport of auxin. Applied P.D.'s may also induce lateral transport, as indicated by electrotropisms, but this is not as yet directly proved.<sup>4</sup> Since light, gravity, and applied P.D.'s do not affect longitudinal transport, it is probable that lateral and longitudinal transport are caused by two entirely unrelated sets of factors.

It is, then, obvious that the P.D.'s measured and discussed in this paper as comprising the plant's electrical polarity are not the cause of polar auxin

<sup>4</sup> BRAUNER and BÜNNING (3) reproduced photographs of electrotropisms in *Avena*. The curvatures are in no way similar to those in photo- or geotropisms, and do not suggest a normal lateral transport of auxin. The applied P.D.'s might induce lateral transport of acid, which would increase the activity of auxin (2) on one side, thus causing the "electrotropism." AMLONG (1), however, indicated some linkage between auxin and electrotropisms, as decapitated roots of *Vicia faba* and decapitated seedlings exhibited no electrotropisms. SATUNSKIJ (C. R. Acad. Sci. U.R.S.S.; n.s. 2: 295-298. 1936) presents additional evidence indicating that auxin might be laterally displaced to the induced anode of coleoptiles in an electrostatic field.

transport. It is still possible that polar transport is effected by a more subtle type of potential gradient not readily amenable to measurement by the methods described above and in a previous paper (5), and that such theoretical P.D.'s are located at phase boundaries or at capillary surfaces (electrokinetic potentials).<sup>5</sup> In the lack of evidence for the existence of the latter type of potential at phase boundaries, etc., in the plant, it is evident that electrical polarity, as described above, and the polar transport of auxin in plants are independent of each other.

### Summary

1. Heteroauxin (indole-3-acetic acid) has a transport number of 0.07 and an absolute mobility of 10.5 mm. per hour under unit potential gradient of 1 v. per centimeter, as measured by conductance experiments.

2. Heteroauxin is electrolytically transported in agar to the anode in an electric field. A potential gradient of 50 v. per centimeter definitely influences the transport. Strictly non-polarizable conditions are necessary in order to establish such an influence.

3. Applied E.M.F.'s have no influence on the longitudinal transport of auxin in *Avena* coleoptiles, although these applied E.M.F.'s reverse or increase the inherent electrical polarity of the same sections.

4. Inverted electrical polarity induced by gravity has no effect on longitudinal auxin transport in coleoptile sections.

5. It is concluded that electrical polarity either has no cause and effect relation to the polarity of auxin transport in the *Avena* coleoptile, or that this relation is not amenable to treatment by the bioelectric methods outlined in this paper. The relation may be real but more subtle than has been revealed by the various types of electrometric measurements employed.

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<sup>5</sup> An example of such a potential gradient might be that in FÜRTH'S (9) "Hochspannungsmodell," in which a potential gradient of several thousand volts per centimeter, produced at a stone electrode, affects dye-uptake in much the same way as tissues concentrate or accumulate substances (10), (11). Another example might be the phase boundary potentials measured by SCHULMAN and RIDEAL (23). These potentials are caused by orientation of polar molecules at interfaces and are measured by electrodes suspended in air above the interfaces, conductivity being produced by ionization of the air by polonium. RIDEAL, in a private communication in 1933, described how a current flow could occur at such interfaces, involving ionic transfer. The details of such mechanisms are discussed by RIDEAL (21).

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# CARBOHYDRATE CHANGES WITHIN THE NEEDLES OF *PINUS PONDEROSA* AND *PSEUDOTSUGA TAXIFOLIA*

CLAIR L. WORLEY

(WITH FIVE FIGURES)

## Introduction

The ability of most coniferous trees and of certain other plants to retain their foliage over several seasons has attracted considerable attention among botanists. This interest is especially keen concerning those plants subjected to extremely cold winters, and which, with rare exceptions, must possess the property of cold resistance, at least during the actual duration of the periods of subzero temperatures. It has long been known, even by the layman, that such coniferous needles are not, in general, resistant to cold during the warmer months of the year.

Consistent studies of the seasonal variations in the physiology of a plant, as well as parallel studies of the variations in the habitat-factors, are necessary to reach an understanding of that species' ability to survive under the environmental conditions representing a given habitat. It has long been recognized that the polysaccharides in leaves, under certain climatic conditions, are converted into the simpler saccharides, and vice versa, by what appears to be the shifting of an equilibrium by means of enzymatic activities. These seasonal variations in the amount and chemical nature of food reserves in the cells of evergreen leaves are in themselves of physiological significance. Although many workers have contributed much important data on this subject, including carbohydrate, osmotic pressure, and pH measurements, our actual information is still fragmentary.

This problem was undertaken to ascertain the quantitative fluctuations of various classes of carbohydrates in *Pinus ponderosa* and *Pseudotsuga taxifolia* needles throughout the more or less dormant season, and to determine a possible relationship between the saccharide fluctuations and the temperature changes.

## Historical data

The measuring of fluctuations in amounts of carbohydrates has been investigated by numerous workers, the majority of whom have limited themselves to diurnal variations when studying photosynthetic tissues. MILLER'S (8) measurements show that the non-reducing sugars in the leaves of most plants are in excess of the reducing sugars, and that the maximum point of increase of the total sugars is also the maximum point of increase of the non-reducing sugars. TOTTINGHAM *et al.* (13) found that the time of day

was less significant than temperature in determining carbohydrate composition; and that solar radiation is the limiting factor in the increases of non-reducing sugars when its value approaches 30° C. In addition to a comprehensive resumé on this phase, CLEMENTS (2) contributed several important pieces of research. He found that starch in the leaf of the sunflower can drop from the maximum to the minimum within one to two hours. The starch content increases after sunset and this, according to CLEMENTS, can be explained only on an actual return of sugars from the petioles. The data which represent the variations in the content of hemicelluloses were of tantamount significance; indicating, as it did, that the part played by these reserves in the metabolism of plants has been generally underestimated.

The seasonal fluctuations in carbohydrates has been investigated for the woody portions of many plants and especially for the apple and the sugar maple trees. There appears to be a quite general agreement among these investigators that starch accumulates in the phloem and the cortex, and perhaps the xylem, of trees in regions of severe winter climate; and in deciduous trees in regions of milder winter climate during the summer and autumn it diminishes to a minimum or disappears in winter, reappears in large quantities in the spring, and diminishes to another minimum as the buds are unfolding. They also found that a relationship exists between starch and sugars; namely, that as the one increases the other decreases, and vice versa. MURNEEK (10), TRAUB (14), HOOKER (4) and MURNEEK and LOGAN (11) have furnished the most important data for apple trees; while JONES and BRADLEE'S (5) paper is the classic and most recent investigation dealing with the sugar maple.

Some work has been completed on seasonal trends of carbohydrates in photosynthetic tissues. SPOEHR (12) in his classic observations on the seasonal variations of carbohydrates in *Opuntia phaeacantha* found that low water content and high temperatures are associated with an increase of pentosans, while a high water content and lower temperatures are associated with a decrease of polysaccharides, an increase of monosaccharides, and a decrease of pentosans. CAMERON (1), while investigating the orange tree, discovered that a different state of affairs occurs in evergreen trees in regions of mild winter climate. His research shows a constant increase in reserves during the autumn and winter. The maximum of reserves, as much as 25 per cent. of the fresh weight, is in the early spring just prior to blossoming. MIYAKE (9), LIDFROSS (6), and others have carried out microscopic observations and have reported that the majority of evergreen leaves of Japan and Europe nearly lost the starch from the mesophyll and the guard cells in winter. The starch content of Japanese coniferous needles is generally more abundant in spring than in late summer or early autumn.

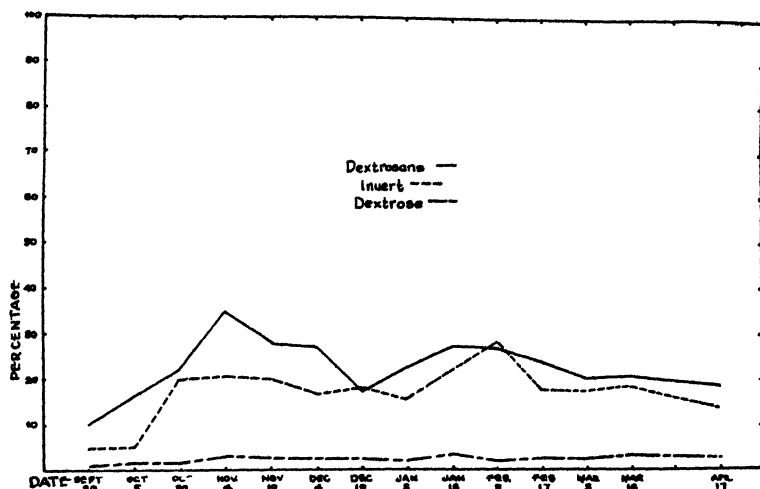


FIG. 1. Percentages of dextrose, invert sugar, and dextrosans occurring in *Pseudotsuga taxifolia* needles when based on fresh weights.

The literature has revealed but one paper closely related to the present investigation. MEYER (7) attempted to find a plausible correlation between physiological changes within the needles and the phenomenon of cold resistance in *Pinus rigida*. His data correspond to that found for the apple and the maple presented above, and, like them, he concluded that the autumnal accumulation of sugars probably results from temperature effects upon the equilibria between soluble and insoluble carbohydrates. This accumulation of sugars during the winter may also be important in the cold resistance of the pitch pine needles through the protective action which sugars exert against precipitation of proteins.

### Material and methods

This study was conducted in the Plant Physiology Laboratory of the University of Idaho at Moscow. The material involved was collected from September, 1935 to May, 1936, and was obtained from the university arboretum immediately south of the campus.

Samples of the needles were taken every fifteen days, at daybreak, just before sunrise. This time of the day was chosen to reduce any variation due to diurnal fluctuations. Needles were always taken from the same trees, and always from the lower branches on the south side. This procedure was an endeavor to obviate, as nearly as possible, such factors as differences in the degree of sunlight, of moisture, and of wind, the relative juxtaposition of neighboring trees, and the individualism of different specimens of the same species.

The needles were brought into the laboratory intact with the branches. If they were moist from dew or rain, it was first necessary to dry them by rolling between paper towels. During the winter months frost, ice, and snow encrusted the needles; and, in such cases, the frozen layer was melted by a current of warm air and dried as above. Approximately 100 gm. of these whole needles were diced into pieces about one-eighth of an inch in length. The diced needles were weighed and recorded as the actual sample. The sample was placed in a wide-mouthed Erlenmeyer flask, in which 0.25 to 0.50 gm. of sodium carbonate had been added to an already boiling medium of 95 per cent. alcohol. Enough 95 per cent. alcohol was used to bring the final concentration of the alcohol to 80 per cent., assuming the fresh weight of the needles to be one-half water. The needles were boiled twenty or more minutes, were covered to a depth of an inch by adding additional 80 per cent. alcohol, and were then stoppered tightly until ready for use.

Owing to the usual difference in temperature outside and in the laboratory it was necessary to complete the above procedure as rapidly as possible to prevent ensuing chemical reactions from altering leaf material. By the method used, the enzymes in the plant tissues should have been almost instantly inactivated. The sodium carbonate, as well as the boiling alcohol, facilitated the destruction because of its alkaline nature and its rapid diffusion into the plant cells. The salt is important in another respect; namely, it neutralizes the various acids which might occur in the needles and which might hydrolyze complex carbohydrates and sugars.

Concomitantly, with the above sampling, determinations of the dry weights were conducted. Duplicate samples—10 to 15 gm.—of homogenous freshly diced needles, taken from the same branches as above, were subjected to 98° C. for two hours to inactivate the enzymes and were then transferred to a vacuum oven at 82° C. to complete the desiccation.

The alcoholic solution was filtered from the stored samples and the residue was washed several times with hot alcohol. The filtrate was again stoppered and stored, while the residue was placed in the vacuum oven to desiccate. This desiccated sample was cooled and weighed, and then ground to a powder fine enough to pass a 100-mesh sieve. Owing to its hygroscopic nature it was necessary to redesiccate in vacuo before weighing the ground residue. The difference in weights represented the amount lost due to grinding, and proved to be an essential factor for the computation of the total polysaccharides.

A Soxhlet extractor was employed to insure complete extraction of the various plant sugars in the ground residue. Seventy per cent. alcohol was used, and it was found, that with this concentration, two and one-half hours' extraction would insure complete removal of sugars from the material.

After extraction, the contents of the extraction flask were added to the original filtrate, and the thimble with its contents was replaced in the vacuum oven to remove all traces of absorbed alcohol. The combined extracts, to which 300 to 500 cc. of distilled water were added, were converted into an aqueous solution by distillation. This aqueous extract was cleared with neutral lead acetate and sodium oxalate. It was filtered into a liter volumetric flask and brought up to volume. The cleared solution was then taken for the determination of the alcohol-soluble free reducing substances. Twenty-five cc. of the cleared solution was used as an aliquot part, which not only insured easy computations but kept the measured amount within the range of available tables.

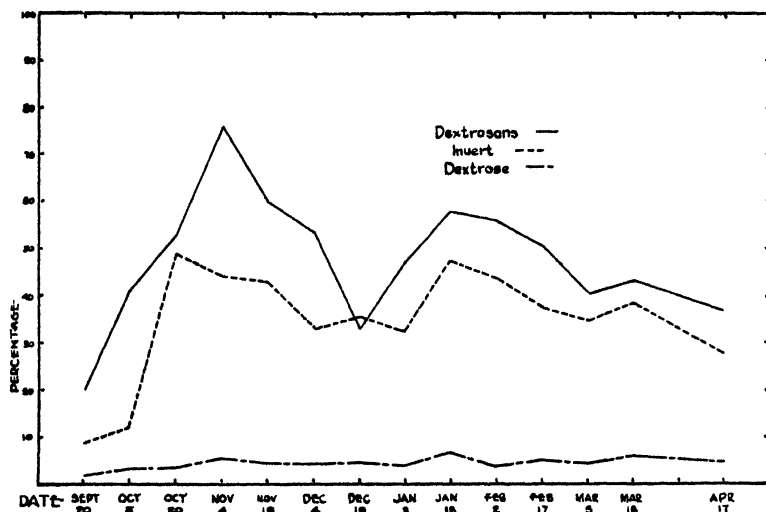


FIG. 2. Percentages of dextrose, invert sugar, and dextrosans occurring in *Pseudotsuga taxifolia* needles when based on dry weights.

To measure the non-reducing sugars (or better, the alcohol-soluble acid-hydrolyzable substances) a 100-cc. aliquot of the cleared solution was used. This aliquot was placed in a 250-cc. Erlenmeyer flask, and 10 cc. of concentrated HCl (s.g. 1.18) were added. Hydrolysis was allowed to proceed for twenty-four hours at room temperatures. At the end of twenty-four hours the acid was neutralized with  $\text{Na}_2\text{CO}_3$  and filtered. The filtrate was put into a one-liter volumetric flask and made up to volume. An aliquot of 25 cc. was taken for reduction to determine the total alcohol-soluble free reducing substances plus the alcohol-soluble acid-hydrolyzable reducing substances.

The total polysaccharides, which included all the alcohol-insoluble acid-hydrolyzable reducing substances, were measured as follows: An aliquot part, approximately 3 gm., of the Soxhlet-thimble residue was used for

hydrolysis. The aliquot was placed in a 500-cc. flask and 100 cc. of water and 10 cc. of concentrated HCl (S.G. 1.18) were added. It was refluxed for two and one-half hours, neutralized, filtered into a liter volumetric flask and made up to volume. A liquid aliquot of 25 cc. was then taken for reduction.

The BERTRAND modification of the MUNSON and WALKER method was used exclusively for the determination of sugars in the above three solutions.

For reasons presented elsewhere in this paper the three classes of carbohydrates were called dextrose, invert sugar, and dextrosans respectively. The total weight of dextrose was computed by determining the amount of copper reduced in an aliquot of the original aqueous solution. In the second instance, the copper reduced was due to the additive powers of the alcohol-soluble free reducing substances and of the alcohol-soluble acid-hydrolyzable reducing substances. Consequently, it was first necessary to subtract the amount due to the dextrose from this total in order to compute the amount due to the acid-hydrolyzable compounds themselves. The computation of the total amount of polysaccharides was more complex, since more factors were involved. First, the amount of copper was transferred to grams of dextrose. Secondly, the weight of dextrose was multiplied by 0.9 to give the amount of dextrosans in each aliquot part. Thirdly, the following formula was employed to convert this into the total amount of polysaccharides in any given sample:

$$\frac{X : 40 \cdot R \ S}{H \cdot G} = P.$$

The figure and letters in the formula have the following significance:

X = amount of dextrosan per aliquot.

40 = the part each aliquot is of the liquid whole.

H = the amount of residue used for hydrolysis.

R = the amount of Soxhlet-extracted residue.

G = the weight of the ground residue.

S = the weight of the original alcohol residue, before grinding.

P = the total weight of polysaccharides in each specific sample.

### Presentation and discussion of data

The chemical data presented herewith in tabular and graphic forms are grouped so as to show most conveniently and clearly the variations in chemical changes of the needles of *Pinus ponderosa* and *Pseudotsuga taxifolia* during the more or less dormant seasons of 1935 and 1936. The data presented portray certain specific tendencies of the carbohydrate metabolism within chlorenchymatous organs.

No qualitative tests were conducted on the three extracts, prior to measurements, to discover the specific reducing compounds concerned. It

seemed feasible to assume that the extracts of coniferous needles would yield reducing substances which were to a greater degree identical with like materials in similar extracts of other plants. Most investigators have like-

TABLE I  
QUANTITATIVE TESTS FOR CARBOHYDRATES IN NEEDLES OF *PSEUDOTSUGA TAXIFOLIA*

DATE	GREEN WT.	DRY WT.	DEXTROSE		INVERT		DEXTROSANS	
			PERCENTAGE OF		PERCENTAGE OF		PERCENTAGE OF	
			GREEN WT.	DRY WT.	GREEN WT.	DRY WT.	GREEN WT.	DRY WT.
	gm.	%	%	%	%	%	%	%
Sept. 20 .....	94.589	49.523	0.91	1.83	4.36	8.80	9.91	20.02
Oct. 5 .....	100.784	39.359	1.29	3.27	4.84	12.30	16.12	40.96
Oct. 20 .....	99.018	40.896	1.53	3.74	19.92	48.70	21.81	53.33
Nov. 4 .....	104.159	46.353	2.68	5.78	20.55	44.32	35.28	76.11
Nov. 19 .....	93.767	46.072	2.25	4.88	19.80	42.96	27.47	59.61
Dec. 4 .....	99.787	50.309	2.17	4.32	16.64	33.07	27.00	53.66
Dec. 19 .....	99.417	50.529	2.32	4.59	17.99	35.59	16.71	33.06
Jan. 3 .....	99.015	47.317	1.94	4.11	13.39	32.53	22.28	47.08
Jan. 18 .....	97.177	46.504	3.21	6.90	22.10	47.53	27.02	58.09
Feb. 2 .....	99.868	47.329	1.89	4.00	20.79	43.92	26.62	56.24
Feb. 17 .....	101.604	46.633	2.55	5.46	17.53	37.57	23.76	50.96
Mar. 3 .....	99.801	49.500	2.31	4.66	17.31	34.98	19.99	40.38
Mar. 18 .....	101.587	47.377	3.04	6.41	18.27	38.56	20.59	43.25
Apr. 2 (No samples)	.....	.....	.....	.....	.....	.....	.....	.....
Apr. 17 .....	99.984	50.583	2.49	4.93	14.08	27.84	18.68	36.83

TABLE II  
QUANTITATIVE TESTS FOR CARBOHYDRATES IN NEEDLES OF *PINUS PONDEROSA*

DATE	GREEN WT.	DRY WT.	DEXTROSE		INVERT		DEXTROSANS	
			PERCENTAGE OF		PERCENTAGE OF		PERCENTAGE OF	
			GREEN WT.	DRY WT.	GREEN WT.	DRY WT.	GREEN WT.	DRY WT.
	gm.	%	%	%	%	%	%	%
Sept. 20 .....	100.410	42.067	0.87	2.07	3.55	8.43	11.69	27.79
Oct. 5 .....	108.583	41.045	0.99	2.41	3.06	7.45	19.43	47.33
Oct. 20 .....	91.127	41.643	2.36	5.66	12.03	36.36	20.20	48.51
Nov. 4 .....	97.237	43.236	2.08	4.81	21.93	50.72	29.71	68.72
Nov. 19 .....	102.602	45.790	2.20	4.81	19.26	42.06	26.53	57.94
Dec. 4 .....	99.638	45.369	2.09	4.61	15.53	34.24	26.31	57.98
Dec. 19 .....	99.844	44.863	2.29	5.11	17.27	38.49	20.55	45.80
Jan. 3 .....	98.740	43.497	2.21	5.09	21.55	49.55	23.56	54.16
Jan. 18 .....	100.472	43.322	2.10	4.85	15.33	35.38	25.67	59.25
Feb. 2 .....	100.189	42.417	2.05	4.83	19.40	45.74	23.89	56.31
Feb. 17 .....	101.634	41.490	2.59	6.25	20.37	49.09	28.17	67.89
Mar. 3 .....	99.715	42.629	3.01	7.07	16.41	38.49	24.05	56.41
Mar. 18 .....	100.752	44.221	2.85	6.45	22.35	50.55	32.66	73.85
Apr. 2 (No samples)	.....	.....	.....	.....	.....	.....	.....	.....
Apr. 17 .....	101.845	44.690	1.65	3.70	12.92	29.45	19.98	45.53



wise assumed that the reducing substances were various sugars without first conducting a qualitative test to authenticate their assumption. This has been a successful assumption to date; however, it leads into an impossibility in the present investigation. Tables I and II show that on November 4, 1935, as well as for other days, the total carbohydrates exceeded the dry weight. Since all previous papers have followed this nomenclature and since it simplifies the phraseology of the discussion, the terms dextrose, invert, and dextrosans will be used, meaning respectively, the alcohol-soluble free reducing substances, the alcohol-soluble acid-hydrolysable reducing substances, and the alcohol-insoluble acid-hydrolysable reducing substances.

It has long been known that a marked difference between gymnosperms and angiosperms occurs not only in the structure but also in the chemical composition of the tissues. The cell sap of conifers has yielded pentose sugars in considerable quantity, and, likewise, the hydrolysis of their cell walls has shown that pentosans constitute the major portion of the structural hemicelluloses. A qualitative test was conducted for pentose sugars and pentosans on November 4 and succeeding dates. Positive results were attained for the dextrose and dextrosan extracts. Their decomposition product, furfural, was detected in both the invert and the dextrosan extracts. Coniferous trees synthesize oil with the approach of cold weather

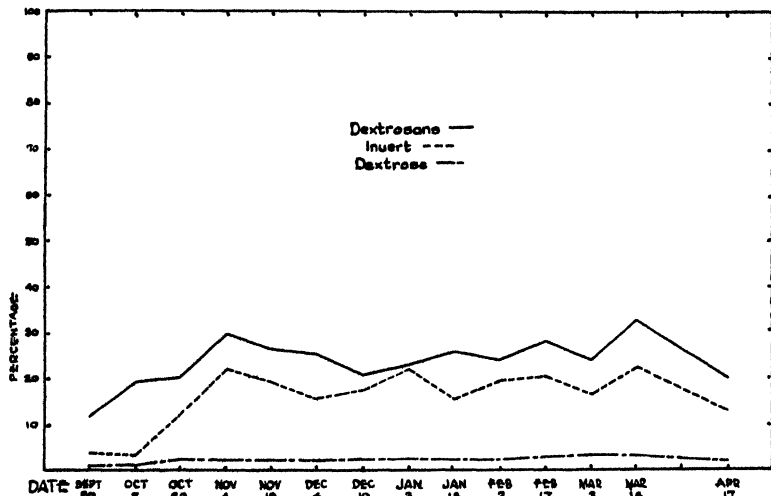


FIG. 3. Percentages of dextrose, invert sugar, and dextrosans occurring in *Pinus ponderosa* needles when based on fresh weights.

in such noticeable quantities that the Germans have frequently referred to them as the "Fettbäume." During this synthesis, hexose sugars are changed into precursors of oils, gums, and fats via pyruvaldehyde and pyruvic acid, both of which will reduce Fehling's solution. If either or both

of these substances were known to be present, or if compounds were known to be present which would on hydrolysis yield these reducing substances, the high amount of cuprous oxide released could be explained. No qualitative tests were conducted for these compounds because of the complicated and lengthy procedures involved. There remains one other plausible explanation; namely, that certain other decomposition compounds with reducing properties occur in the extracts. That this last actually did occur has been partially proven. Formaldehyde was detected in the dextrosan extract on November 4 for both species. Secondly, the sudden drop in temperature the first few days of November, prior to the establishment of the needles' cold-resisting mechanism, injured the needles of conifers throughout the arboretum. They became brown, flaccid, and underwent abnormal senescence. Some rotted on the limbs, while others dropped throughout the winter. This specific condition is abnormal to prevailing conditions in the Pacific Northwest. Although needles with macroscopic injuries were avoided, it does not prove that the samples were void of material with internal injuries and decomposition products.

Repeated checks have definitely proven that the fallacy is not in the procedure and methods involved but must be incorporated in the interpretation of the data secured. The various extracts, and especially the dextrosan, must therefore contain other reducing compounds in addition to the sugars.

Considerable care should be taken before drawing conclusions from the data based on percentages. The order of error, under the conditions here measured, seems about the same whether expressed as percentage of dry weight or of fresh weight if, on the one hand, the change is due to dry matter loss, and, on the other, to water absorption.

Attention is called to the low percentages and the limited variations in the dextrose during the colder seasons. Figures 1 and 3 illustrate this uniformity, which would be even more striking if based on its divergence from a seasonal average. That dextrose remains a fairly uniform constituent part of the coniferous needles is in accord with results obtained by JONES and BRADLEE (5) and others. These results, however, are not universal, for MURNEEK and LOGAN (11), HOOKER (4), and TRAUB (14), all of whom worked with the apple, found significant variations of these reducing sugars throughout the year's cycle; however, even they found a marked uniformity during the colder seasons. MEYER'S (7) results on the pitch pine leaves showed a limited variation in dextrose from October to April. All of the above data point to the lability of dextrose solutions, which indicates that it might be an intermediate product involved in the metabolism of the plant. Undoubtedly, upon reaching a certain maximum percentage, dextrose is condensed or polymerized into reserves and structural materials of the

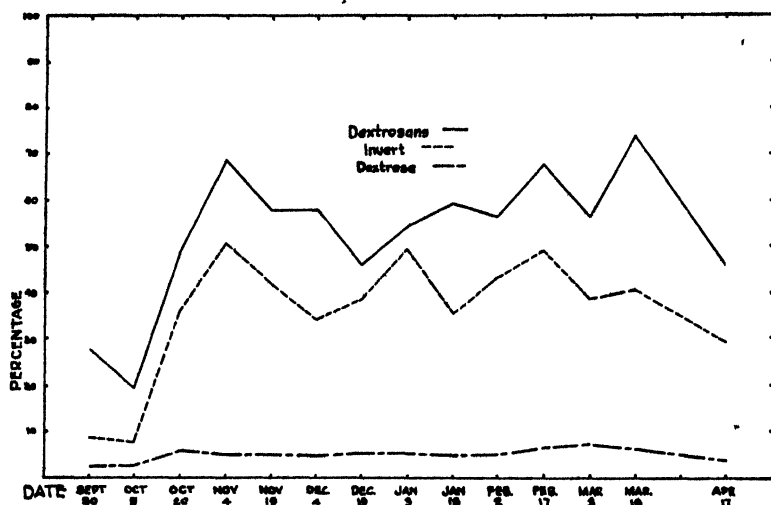


FIG. 4. Percentages of dextrose, invert sugar, and dextrosans occurring in *Pinus ponderosa* needles when based on dry weights.

plant. Catabolic processes, on the other hand, would tend to produce dextrose as a hydrolysis product of the reserves and as an intermediate stage in the plant's respiratory functions. Many have assumed that environmental factors do not affect variations in dextrose percentages directly, but only indirectly through their influence on photosynthesis and hydrolysis of reserves on the one hand and on respiration and the synthesis of reserves and structural materials on the other.

Figures 2 and 4 tend to accentuate the great variations in invert sugars throughout the colder seasons. No maximum was found in midwinter, as has been reported in numerous papers. This at first appears contradictory but, on a more detailed study, agrees in its entirety with the results of the above workers if based on temperature relationships.

It is, in general, to be noted that invert sugars undergo no periodic changes but necessitate the establishment of the nature and the rate of its metabolic rearrangements and disintegration as affected by the climatic conditions. CLEMENTS (2), working with the sunflower, and other investigators working with the apple and the maple, have reported actual migration of sucrose from the branches and petioles back into the leaves on the approach of cooler weather. This in itself would partially explain the relatively high constituent part of invert sugar in the October 20 sample, for both the pine and the fir. The high results of November 4 and thereafter are, however, probably due to the presence of certain decomposition products.

The gradual lessening of the starch content, and other polysaccharides, in other plants from September on, in opposition to the accelerated increases of the sucrose and the hexoses which are maintained throughout the winter, has been recorded in all research papers reviewed, save one. That a different state of affairs obtains in evergreen trees in regions of mild winter climate is indicated by CAMERON's (1) investigation, which shows a constant increase in reserves during the autumn and winter. He obtained a maximum in early spring just prior to blossoming when the reserves constitute as much as 25 per cent. of the total fresh weight. MEYER (7), the only other worker on the carbohydrates in coniferous needles, did not measure the polysaccharides. The data represented by tables I and II or by figures 1 and 3 clearly reveal that the dextrosans did accumulate during autumn and did not decrease as recorded by most investigators. The higher peaks plotted require an explanation based upon interconversion into different types of compounds rather than upon actual storage of reserves. It is of interest to note that a definite relationship does not exist between the dextrosan and the invert sugar curves. For *Pinus ponderosa* from September 20 to October 5 and from December 4 to January 18, inclusive, and for *Pseudotsuga taxifolia* from December 4 to January 3, inclusive, the dextrosan curves increased and decreased concomitantly with the opposite accelerated change in the invert curves. At all other times a change in the one class was accompanied by a similar change in the other. There were no relative changes found in either case, and therefore the data do not authenticate a direct relationship between dextrosans and invert sugars. They do, nevertheless, indicate a possible relationship for the dates listed above, but even in these cases there is no correlation.

A marked modification of the starch content concomitant with changes in the temperature was observed by LIDFORSS (6), who showed that all European evergreen leaves in temperate latitudes are quite starch-free from the beginning of December throughout the winter. With the advent of higher temperatures in spring the starch again appears. MIYAKE (9) reported similar conditions existing in Japan. In the present investigation, the Soxhlet-residue was treated with iodine and examined microscopically. The results are tabulated in table III. Only once, on November 4 immediately following the sudden drop in temperature, was the Douglas fir void of starch. The data, taken as a whole, do agree with the results of the above papers; namely, the relative amount of starch decreases with a decrease in temperature and increases again with a return to higher temperatures. It is worthy of notice that with a change in relative amounts of starch there is often the opposite change in the quantity of dextrosans. This materially strengthens the hypothesis that the changes occurring in the dextrosan

TABLE III  
RELATIVE AMOUNTS OF STARCH OBSERVED MICROSCOPICALLY\*

DATE	<i>PSEUDOTSUGA TAXIFOLIA</i>	<i>PINUS PONDEROSA</i>
Sept. 20 .....	Medium	Slight
Oct. 5 .....	Slight	Medium
Oct. 20 .....	Slight	None
Nov. 4 .....	None	None
Nov. 19 .....	Medium	Slight
Dec. 4 .....	Medium	None
Dec. 19 .....	Slight	None
Jan. 3 .....	Slight	Slight
Jan. 18 .....	Slight	None
Feb. 2 .....	Slight	Slight
Feb. 17 .....	Medium	Slight
Mar. 3 .....	Medium	Much
Mar. 18 .....	Much	Medium
Apr. 2 .....	.....	.....
Apr. 17 .....	Abundant	Abundant

\* An arbitrary set of standards was employed.

extracts are due to changes in the types of reducing substances rather than actual changes in the quantity of reserves present.

An attempt was made to determine a possible correlation between saccharide fluctuations and climatic changes.

Meteorological data covering the entire period of this study were secured from the local Weather Bureau situated within an eighth of a mile of the arboretum where the trees were located.

Little doubt exists that certain metabolic functions within the plant are regulated by climatic changes indirectly. It is equally agreed that, of all the climatic factors, temperature is the most potent in its regulatory effect on the changes in carbohydrate quality. The influence of temperature on the carbohydrate content of plants such as conifers is, of course, the summation of the effect of this factor on a variety of activities; and, as a consequence, no mathematical correlation is to be expected. The data bear this out on the one hand, while on the other they establish certain relationships with the specific types of the elaborated foods. Since there are numerous ways by which temperature data can be represented, they were graphed in various forms to determine which most closely agreed with the carbohydrate data presented herewith. The curves were plotted from averages of the five days prior to each sampling. Whether the minimum temperature is the more important in its effect on certain enzyme actions causing the conversion of reserves into labile forms, or whether the maximum temperature in its regulatory rôle on respiratory and photosynthetic activities is the more important, is very difficult to ascertain.

A comparison of figure 5 with figures 1 and 3 obviates the necessity of detailed discussion and demonstrates the generalizations below. The dex-



FIG. 5. Maximum and minimum temperature curves computed from averages of five days prior to each sampling.

trose content starts at a relative minimum and increases toward a maximum, which is limited to a small percentage of the total fresh weight, as the minimum temperature drops toward the freezing point. During the freezing temperatures the dextrose percentage remains comparatively near its maximum and fluctuates only slightly. This is strengthened by the results of JONES and BRADLEE (5), which tend to demonstrate that the so-called critical tree temperatures need not cover a very wide range above or below 32° F. within the tree and are governed by corresponding external air conditions.

The distinct gain in invert storage in the autumn months, which is shown with its maintenance, though in different proportions, throughout the winter months, does indicate a definite relationship with the prevailing temperatures. A superimposition of the invert sugar curves upon figure 5 definitely reveals an inverse relationship. This is universally accepted.

Except for CAMERON (1), all investigators have reported a decrease in percentage of reserve foods coincident with a lowering of the temperature and a redeposit of these carbohydrates with a return to the higher temperatures. Table III when compared with figure 5 shows that this relationship for starch holds for the coniferous needles concerned. The dextrosan curves, represented in figures 1 and 3, however, agree with similar measurements by CAMERON (1), on the orange. The sharp maxima in the curves can be explained only by decomposition products caused by the very sudden drops in the temperature during the days just prior to the taking of

the samples. A definite positive relationship between starch content and temperature appears to exist; while a negative relationship, just as striking in aspect, is evident between erratic temperature changes and the fluctuations in alcohol-insoluble acid-hydrolyzable reducing substances. When the temperature changes were gradual a positive relationship existed between temperature and this class of compounds.

Other habitat factors, although exerting less effect, might be considered. From data not included here, it was found that the range of available sunlight presented no measurable fluctuations. A similar comparison indicates that the water content may either diminish or accentuate the effect of the temperature. This depends whether a drop in the former is coincident with a drop in the latter or if a rise in the former and a lowering of the latter occur at the same time.

It was found by GAIL (3) that the osmotic pressure of expressed cell sap in some deciduous and non-deciduous plants reached a maximum during the winter months, and that there was a decrease in the spring and a gradual rise again with the arrival of autumn. He advocated the theory that this change of osmotic pressure was partially due to the changes in the sugar content. A plausible connection between variations in carbohydrates and osmotic pressure was sought; but, since no osmotic pressure measurements were made on the cell sap of the material involved, it was necessary to seek the relationship through an indirect procedure. It is possible, however, to compare GAIL's data and the data of this study by using the respective relationships with the temperatures as the medium. From such a comparison it is possible, and may be argued, as MEYER (7) has likewise pointed out, that the invert sugars in the needles serve to regulate the osmotic pressure owing to its ready interconversion to other forms. This regulation could be quite as well effected by the precipitation of the polysaccharides.

### Summary

1. Quantitative measurements were made for three classes of carbohydrates in the needles of *Pinus ponderosa* and of *Pseudotsuga taxifolia* to determine their fluctuations during the more or less dormant seasons. The BERTRAND modification of the MUNSON and WALKER method was followed.

2. Reducing compounds other than sugars occurred in some of the extracts. Furfural and formaldehyde were definitely identified.

3. The hexose and pentose sugars, or better, the alcohol-soluble free reducing substances, maintained low percentages and limited variations during the colder seasons.

4. The distinct gain in alcohol-soluble acid-hydrolyzable reducing substances in the autumn months, with its maintenance, though in different proportions, throughout the winter months, indicates a definite inverse re-

lationship with the prevailing temperatures. Disaccharides and furfural are the major constituents of this group of compounds.

5. Alcohol-insoluble acid-hydrolyzable reducing substances, which contain starch, other dextrosans, and reserve hemicelluloses, increased rapidly immediately following a sudden drop in temperature; during gradual temperature changes a positive relationship existed.

6. The relative amount of starch decreased with a decrease in temperature and increased again with a return to the higher temperatures.

7. The changes occurring in the "dextrosan" extracts are due to a change in the type of reducing substances rather than an actual change in the quantity of reserves present.

8. The seasonal variations in the needles' soluble carbohydrate content, including hexoses, pentoses, and certain hemicelluloses, appear to be partially responsible for the seasonal variations in the osmotic pressure of the cell sap.

The writer wishes to express his gratitude to Dr. FLOYD W. GAIL for suggesting this problem and for his guidance throughout the investigation and preparation of the manuscript.

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# SURVIVAL OF ISOLATED TOMATO ROOTS AT SUBOPTIMAL AND SUPRAOPTIMAL TEMPERATURES

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(WITH ONE FIGURE)

In their early root-tip cultures, ROBBINS (3, 4) and ROBBINS and MANEVAL (5, 6) regularly made transfers to fresh media at about 2-week intervals. No reason is given by the authors for the choice of this period. KOTTE (1) and MALYSHEV (2) used periods of various lengths, determined apparently by the increment rates of the individual cultures. In earlier work (7, 8), dealing with the effects of various external factors on growth of isolated roots, three components of the environmental complex: passage length, concentration of sugar, and concentration of yeast extract, were arbitrarily chosen to correspond with those used by ROBBINS. Since only the gross increments for the entire period were recorded, this passage length appeared to be satisfactory. In later work, however (9) when increments were recorded daily over an extended period of time instead of fortnightly, it became evident that the interval previously chosen was far too long for optimal results. The mean daily increments for 100 cultures measured daily for 25 weeks were, for the first 7 days: 3.6, 4.2, 5.6, 6.1, 5.9, 6.4, and 5.8 mm. per culture per day. The maximum rate of growth was thus reached somewhere between the 4th and 6th days. Thereafter the growth rates were not recorded regularly, but it was noted that they fell off gradually until, at about the 10th to 12th day, the increments were negligible. In most of the experiments subsequently carried out with these cultures, a period of one week, slightly longer than the optimum indicated by these figures, was employed, since such a period simplified the keeping of records.

A routine involving weekly transfers is satisfactory where, as is the case in most experimental work, a rapid turnover of cultures is desirable. However, when a great number of cultures has to be maintained, weekly transfers become costly of both materials and time. Such is the case, for example, with the group of stock cultures of virus-bearing roots maintained in this laboratory (10). A half-dozen different viruses, each in a separate set of roots, and each requiring a minimum of 10 replications for its proper maintenance, makes a total of 60 cultures. It is anticipated that ultimately many more viruses will be maintained in root cultures.

Although optimal growth did not ordinarily occur at room temperature after the 6th day, cultures were in some cases kept alive over very much greater periods of time without transfer. Roots left for long periods with-

out transferring ceased to grow normally; the growing points became swollen and crooked, and often turned brown. After a period of dormancy, new branch roots were put out, often in clusters just back of the old growing points, and active growth was resumed for a time. These new rootlets finally stopped growing, the same symptoms of injury appeared, and a second period of dormancy supervened, followed by the formation of still another set of new growing points. Such a cycle might be repeated several times, the cultures remaining alive even after the culture solution had evaporated down to a small fraction of its original volume. Cultures of clone B (9) were recovered on one occasion after being allowed to stand without transfer for 145 days. Cultures so treated, however, were subjected to considerable danger of ultimately becoming contaminated by penetration of molds through the cotton plugs, and, although roots so infected were frequently recovered in an aseptic condition, the risk of loss was great. Moreover, when a root was allowed to grow undisturbed for a long period, even though the growth rate was definitely suboptimal, the flask became filled with a tangled mass of branching rootlets from which it was difficult to isolate and remove satisfactory fragments for subculture. The handling involved in the isolation of subcultures from such masses greatly increased the danger of loss by contamination. Furthermore, recovery of such material upon transfer to fresh nutrient was in a considerable percentage of cases slow and irregular. This procedure was obviously not to be relied upon as a routine method of maintaining cultures for long periods.

With these observations in mind, it seemed worthwhile to attempt to develop procedures by which the length of time between transfers could be increased without seriously impairing the vitality of the roots. The temperature-growth-rate curve for isolated tomato roots has previously been shown (11) to be extremely sharp, with a maximum at 30° C. Since growth was reduced both above and below this point, the use of suboptimal and supraoptimal temperatures seemed to offer a possible means of increasing the passage length. The present paper describes experiments planned to deal in greater detail with the effects of such temperatures.

### Experimentation

As was shown elsewhere (11), growth of isolated tomato roots was practically *nil* at 5°, 8°, 10°, and 40° C., and took place at a very slow rate at 15° and 35° C. Growth at temperatures between 20° and 33° C. was fairly rapid and could be maintained indefinitely, but at 32° and 33° C. the roots were often discolored and evidently in poor condition. To determine whether the submaximal growth rates obtained at suboptimal and supraoptimal temperatures were due to injury or merely to temporary retardation of the metabolic processes, cultures kept for one week at each of the

temperatures mentioned above, with the exception of 32° and 33° C. were subsequently transferred to room temperature (*ca.* 22° C.) for a second week. Growth rates were recorded for both passages. In all cases, with the exception of those kept at 35° and 40° C., the growth rates at 22° C. were normal, that is, recovery was complete (fig. 1). Cultures grown at 35° and 40° C. and then transferred to 22° C., on the other hand, made no recovery.

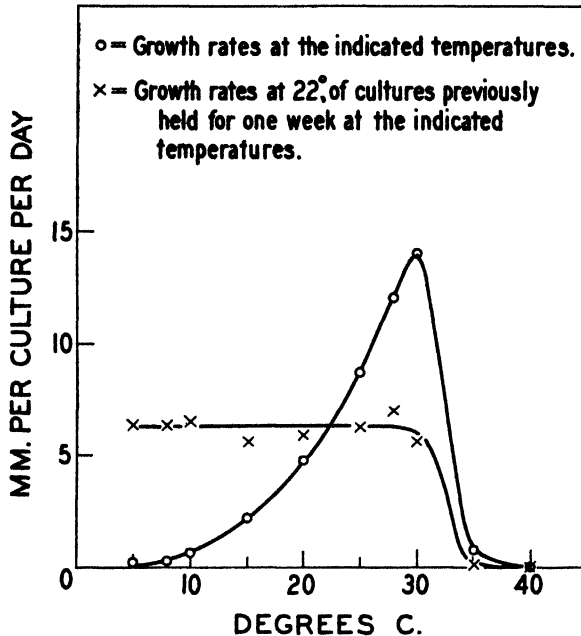


FIG. 1. Growth rates of isolated tomato roots grown for one week at a series of controlled temperatures and then transferred for a second week to a temperature of about 22° C.

It was evident that, while brief exposure to suboptimal temperatures not reaching the freezing point was not seriously detrimental to isolated roots, similar exposure to supraoptimal temperatures did seriously injure them. Since it is known (11) that continuous growth at a moderate level can be maintained at 20° C. (winter room temperature), further study was restricted to temperatures below this level. Growth was studied in detail at two temperatures, about 8° and 15° C.

Survival at about 8° C. was tested over periods of from 1 to 10 weeks (March 5 to May 14, 1934). One hundred cultures from clone C (9) were prepared simultaneously and measured. They were placed together in a darkened cellar room having at the beginning of the experiment a temperature of 6.7° C. and at the end of the 10-weeks' period a temperature of 16° C. The highest temperature reached while viable roots remained in the

room (see below) was 11° C. The gradual rise in the temperature was due to the coming of spring. Ten cultures were removed at the end of each week to a laboratory having a temperature of about 22° C. and were measured. They were allowed to grow and were transferred each week. This was continued until either they had resumed a rate of growth equal to that in the controls, or else had shown clearly that they would not attain such a growth rate within a period suitable for routine use. The approximate growth rates of this series expressed as percentages of the controls are shown in table I. After the first week growth at 8° C. was too little to be measured.

TABLE I

INCREMENT RATES OF ISOLATED TOMATO ROOTS GROWN FOR VARIOUS PERIODS AT 7-16° C. AND THEN TRANSFERRED TO 22° C.

		TEM- PERA- TURE	WEEKS→	1	2	3	4	5	6	7	8	9	10
	0	7		100	100	100	100	100	100	100	100	100	100
Weeks	1	7		5	121								
	2	8		5	0	40	148						
before	3	8		5		0	0	0	120				
	4	10		5			0	0	95	140			
transfer	5	11		5				0	0	6	110		
	6	11		5					0	0	0	0	0
to	7	12		5						0	*	-	-
	8	13		5							*	-	-
22°	9	15		5							*	-	-
	10	16		5							*	-	-

\* Contaminated with molds.

Recovery of each group as a whole was complete after 1, 2, 3, 4, and 5 weeks. At the end of this time the temperature of the culture room was 11° C. Recovery did not occur when cultures were exposed for longer periods. After one week, 9 of the 10 cultures resumed growth from the old growing points. The same was true after 2 weeks. But after 3 weeks all original growing points were dead and the subclone was recovered from newly laid down lateral initials in 3 cultures which survived out of the original 10. After 4 weeks, 4 cultures survived and produced viable laterals, but were without viable terminal growing points. After 5 weeks, 3 cultures survived in the same way. After 6 weeks, 2 cultures survived but grew very poorly. Seven weeks after beginning the experiment, all cultures in the experimental room became simultaneously contaminated with molds. This general contamination was probably due to the excessively moist conditions prevailing in the room at this time, which allowed molds to develop on and penetrate the cotton plugs protecting the flasks.

It is evident from the data presented above that, although recovery *may* occur after 5 or 6 weeks at 7–11° C., such recovery is uncertain after more than 2 weeks. Temperatures of 10° C. or less thus offer little if any advantage over room temperature for the maintenance of roots.

Survival at 15° C. was tested in the same way, using a constant temperature oven controllable to  $\pm 2.0^\circ$ . Eighty cultures were prepared and 10 cultures removed after each week for 8 weeks. The results are shown in table II. The mean growth rate for all cultures at 15° C. was 1.6 mm. per

TABLE II  
INCREMENT RATES OF ISOLATED TOMATO ROOTS GROWN FOR VARIOUS PERIODS  
AT 15° C. AND THEN TRANSFERRED TO 22° C.

WEEKS→	1	2	3	4	5	6	7	8	9	10
0	100	100	100	100	100	100	100	100	100	100
Weeks 1	30	164	147							
at 2		30	106	147						
15° C 3			30	83	140					
before 4				30	43	120				
transfer 5					30	80	163			
to 6						30	146	156		
22° 7							30	150	159	
8								30	123	197

culture per day, about 30 per cent. of that of the control. At every period studied the roots showed some recovery in the first week after exposure to room temperature, and in only three cases (3, 4, and 5 weeks) were the experimental cultures exceeded by the controls. When held for 2 weeks at room temperature, all experimental groups recovered completely, and at the end of this time all were growing *more* rapidly than the controls. The mean percentage index for all groups was 153, and the lowest index, that for 4 weeks at 15° C., was 121. It is thus evident that such roots could not only be kept without transfer for at least 8 weeks at 15° C. without injury, but that they were actually stimulated by such treatment.

These figures, however, do not tell the whole story, for the original growing points of all cultures kept for more than 5 weeks at 15° C. were killed, recovery in these cases being from newly formed laterals. Symptoms of injury similar to those occurring at room temperature after 10 to 12 days appeared in these cultures after 4 to 5 weeks.

It seems probable from these results that the temperature at which truly satisfactory growth can be maintained with the greatest length of passage should lie somewhere between 8° C., at which no growth occurred, and 15° C., at which the growth rate was reduced to about 30 per cent. of its value at room temperature. For practical purposes, however, a more exact analysis is unnecessary. By keeping cultures at 15° C., isolated roots can be

maintained in good condition without transferring oftener than once in 2 months. The application of such a method in the maintenance of stock cultures is evident.

### Summary

Isolated tomato roots kept at 7–11° C. for longer than two or three weeks without transfer die. If kept at 22° C. they must be transferred weekly if they are to be maintained in good condition. At 15° C. they will remain in good condition if transferred at intervals of two months. A temperature of about 15° C. is thus more suitable than 8° C. or 22° C. for the maintenance of large numbers of stock cultures.

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# SEPARATION FROM YEAST OF MATERIALS ESSENTIAL FOR GROWTH OF EXCISED TOMATO ROOTS

PHILIP R. WHITE

(WITH ELEVEN FIGURES)

## Introduction

Three years ago the results of experiments on the cultivation of excised tomato root-tips, *in vitro*, for a period of one year were reported (7). From the data available at that time it was concluded that such roots could be kept growing and be maintained in apparently normal condition for potentially unlimited periods of time. The environmental complex used was shown to be adequate for all requirements of growth. Aside from possible traces of impurities in the salts and sugar used, only one factor of the environmental complex, namely, the extract of yeast, amounting to 0.01 per cent. of the nutrient, was recognized as unknown. Identification of the materials in this unknown and their replacement by known materials were considered important problems for the future. The idea that any growth-limiting material, such as a hormone, might be furnished to the culture from the parent plant was shown to be untenable. Since, however, such diverse materials as vitamins, auxins, auximones, bioses, and nitrilites, as well as biologically active sugars, accessory mineral elements, haemin, sex hormones, insulin, nucleic acids, porphyrins, and many other materials of uncertain physiological significance have been recorded as constituents of yeast, the presence of the yeast extract in the nutrient obviously represented a serious stumbling block to any definite conclusions as to the nutritional requirements of the plant tissues under consideration. The fact that ROBBINS (2) using a nutrient-containing peptone, WHITE (4) using a fibrin digest, KOTTE (1) and WHITE (4) using meat extract (LIEBIG), and KOTTE (1) using various amino acids, all obtained some growth, suggested that the constituents necessary for normal growth might not be specific to yeast, but none of them had been identified. The work presented here was planned to throw light on the nature of these constituents.

## Materials and methods

Roots for study were of a single clone obtained from the tomato variety Bonny Best. They had been kept in continuous culture for more than two years (8). All tips used were selected carefully for uniformity in appearance. Successive series of 14 to 16 different nutrient solutions, each represented by 20 cultures, were grown under as uniform conditions as were obtainable in an ordinary diffusely lighted laboratory. Each series, unless



otherwise, stated, included two sets of controls one employing the standard nutrient developed in previous work (5, 6), the other the same nutrient with the yeast extract omitted. The results obtained in the 12 to 14 nutrients under investigation in each series were then compared with those given by one or both of the controls.

The method of preparing the standard nutrient (6, 7) has been somewhat modified. The constituents were made up in five solutions containing: (1) nutrient salts, (2) "accessory" salts, (3) iron, (4) sugar, and (5) accessory organic materials. The nutrient salt solution was prepared as follows: 140 gm.  $(\text{CaNO}_3)_2 \cdot 5\text{H}_2\text{O}$ , 67 gm. KCl, 81 gm.  $\text{KNO}_3$ , and 12.25 gm.  $\text{KH}_2\text{PO}_4$  were dissolved in 8 liters of distilled water. 74 gm.  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  were dissolved in 2 liters of water. The second solution was then poured slowly into the first with constant stirring. A slight precipitate eventually formed but was not carried over into the final nutrient. Ten ml. of this stock solution were sufficient for a liter of nutrient. The "accessory salts" solution contained 300 mg.  $\text{ZnSO}_4$ , 270 mg.  $\text{Na}_2\text{SiO}_3$ , 1070 mg.  $\text{Al}_2(\text{SO}_4)_3$ , 150 mg. KI, 320 mg.  $\text{H}_3\text{BO}_3$ , 560 mg. NaCl, 440 mg.  $\text{MnSO}_4$ , 40 mg.  $\text{NiCl}_2$ , 39 mg. LiCl, 40 mg.  $\text{CoCl}_2$ , and 10 mg.  $\text{CuSO}_4$  in ten liters of water. As in the case of the nutrient salt solution, 10 ml. of this solution were sufficient for 1 liter of nutrient. The iron and sugar were made up fresh each time nutrient was prepared; 2.5 mg.  $\text{Fe}_2(\text{SO}_4)_3$ , and 20 mg. sucrose were added to each liter of nutrient. The accessory organic materials were the only variables in the experiments to be reported here. In the nutrient used for cultures designated as "controls without yeast," such materials were entirely omitted. The "controls with yeast" contained yeast material prepared as follows: 25 mg. of yeast ("Brewer's Yeast—Harris") were boiled for  $\frac{1}{2}$ -hour in 2 liters of distilled water. While still hot the preparation was centrifuged for 5–10 minutes at high speed. The supernatant fluid was decanted, made up to 2.5 liters and distributed to Pyrex test-tubes in 50-ml. aliquots. These were autoclaved at one atmosphere pressure for 20 minutes, cooled, and stored for future use at  $-15^\circ \text{C}$ . Ten ml. of this preparation were sufficient for 1 liter of nutrient. One liter of the standard control nutrient then contained 10 ml. of the nutrient salt solution, 10 ml. of the accessory salt solution, 10 ml. of the yeast preparation, 2.5 mg.  $\text{Fe}_2(\text{SO}_4)_3$ , and 20 gm. sucrose.

The roots were measured daily, and the growth increments of the 20 (sometimes 10) cultures in each nutrient averaged. Most series were repeated two or more times. The results have been plotted as average increments in length expressed in terms of millimeters per culture per day. The curves obtained indicate the increment trends clearly. The experiments reported were made during the period from September, 1934, to May, 1936.

### Experimental results

Yeast extract was shown by ROBBINS (2) and WHITE (5) to be beneficial to isolated root-tips. ROBBINS found that the stimulative effect did not become evident until after several passages (2). WHITE's experiments with yeast were carried out for a single passage only and were, therefore, unsuitable for determining any but the gross requirements of the tissue. Moreover, the measurements recorded by both authors were made only at the end of one or more passages of 2 weeks' duration. It seemed possible that the growth recorded might all have occurred in the first part of the passage, at the expense of material from the tissue of the initial explant. Thus, neither author demonstrated unequivocally the essential nature of these yeast materials. To determine whether or not records of daily growth rates would throw light on this problem, 10 cultures were made in a nutrient lacking yeast material, with 10 controls in a complete nutrient. The increments were recorded daily for one week. The results are plotted in figure 1. The average *total* increment in the first passage, in the absence of yeast, was 41 per cent. of that obtained in the control nutrient, a figure comparable to that previously recorded by ROBBINS (2) and by WHITE (7); but the average *final* daily increment rate in the absence of yeast was only 13 per cent. of the control, indicating that very little growth was possible as the end of the period was approached. Subcultures were made from each set to a nutrient similar to that used during the first week. The growth rate of the experimental cultures decreased still further, so that at the end of the second passage the increments at the original growing points in the experimental series were *nil* and the growth that occurred in the most rapidly growing branches was only about 2 per cent. of that recorded for the controls. Addition of yeast ash (3) on the 5th day of the second passage did not increase growth.

The slow rate of increment at the end of the second passage is not, however, indicative of lack of vitality in the cultures. More branches were formed in the absence of yeast than in its presence. But these branches, although in apparently healthy condition, grew for only a short time. The tips soon died, and new branches were formed. This suggested that essential nutrients, freed by the dying of the older growing points, were reutilized in forming new ones. When cultures grown in a deficient nutrient were transferred at the end of the second passage to a complete nutrient, the increment rates rose sharply on successive days from 0 per cent. to 46, 91, 110, 140, and 180 per cent. of the controls. In the fourth passage, this high level was at first maintained but fell off gradually until in the fifth passage both groups were growing at approximately the same rate. Similar behavior was observed when roots were subjected to sub-optimal temperatures (7–10° C.) for a time and then returned to room temperature (9). This observation seems to indicate that adverse conditions may slow down certain meta-

bollic processes while leaving others unaffected, permitting storage of excess quantities of some material which under normal growing conditions is a

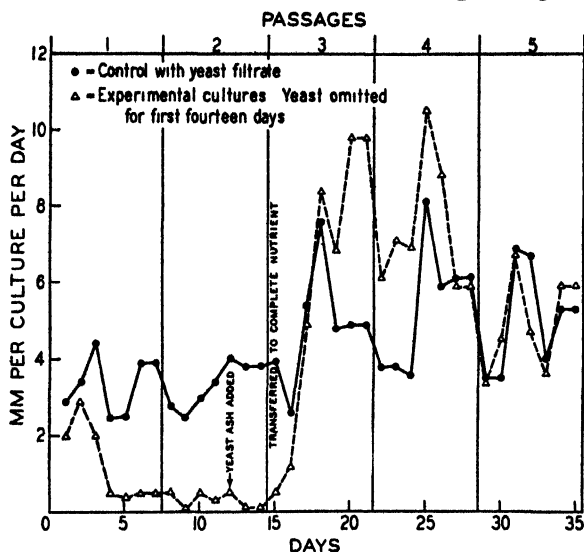


FIG. 1. Effect of omission of yeast extract from the nutrient for 2 passages,\* followed by return to a complete nutrient for 3 passages thereafter.



Photograph by J. A. CARLILE

FIG. 2. Upper row, roots grown for 3 weeks in nutrient lacking yeast extract. Lower row, roots grown without yeast extract for 2 weeks, then returned to a complete nutrient for 1 week.  $\times 0.5$ .

\* "Passage 1" in this experiment is passage 76, counting from the time when the clone was isolated. The roots had been maintained through 75 passages under standard conditions before being used in this experiment. Other experiments recorded here were carried out with roots previously maintained for from 75 to 160 passages under standard conditions.

limiting factor. In further experiments roots kept in a nutrient lacking yeast for periods of 1, 2, 3, 4, and 5 weeks all recovered when transferred to a complete nutrient. Examples of such roots are shown in figure 2.

The results indicate clearly that some material supplied by yeast extract is essential for satisfactory growth. The rapidity with which this deficiency shows up, as indicated by the abrupt divergence between the curves of figure 1, shows that this material is carried over with the explant in smaller amounts than are required to sustain normal growth over a period of one week or longer. These results have been verified repeatedly in the controls of a number of different experiments. It is evident that under the experimental conditions used here, the form of the growth curve within a single passage can be taken, tentatively at least, as an indicator of the efficiency of any nutrient.

A standard concentration of an extract of 100 mg. dried Brewer's yeast (Harris) per liter of nutrient was used in all experiments up to this point. The choice of concentration to be used was based on the work of ROBBINS

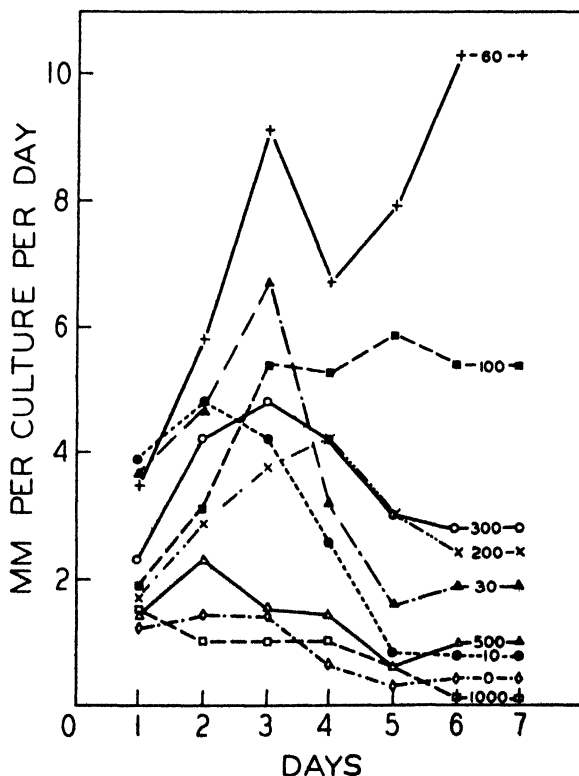


FIG. 3. Curves showing growth rates in nutrients containing extracts of from 10 to 1000 mg. of yeast per liter.

(2), and was about the minimum which he found satisfactory. Since the yeast employed by ROBBINS was autolyzed, while the preparation used in this work was pasteurized at the height of its activity so that only the free, water-soluble materials were available, it seemed desirable to determine the concentration of these materials that would prove optimal for growth. Nutrient solutions containing 10, 30, 60, 100, 200, 300, 500, and 1000 mg. of yeast per liter were therefore tested. The results are shown in figure 3. They indicate that the standard concentration of 100 mg. of yeast per liter, employed in the past, was close to the optimum, being exceeded by only a single concentration, 50 mg. per liter. This concentration has, therefore, been retained for all subsequent work.

Since yeast does evidently furnish some material or materials essential for growth of tomato root tips, and the optimal concentration of the raw extract was known, an attempt was made to isolate this material or materials by various types of fractionation.

#### SOLUBILITY IN WATER

To determine whether or not the growth-promoting material was completely extracted by boiling water, 50 gm. of yeast were boiled for  $\frac{1}{2}$  hour in

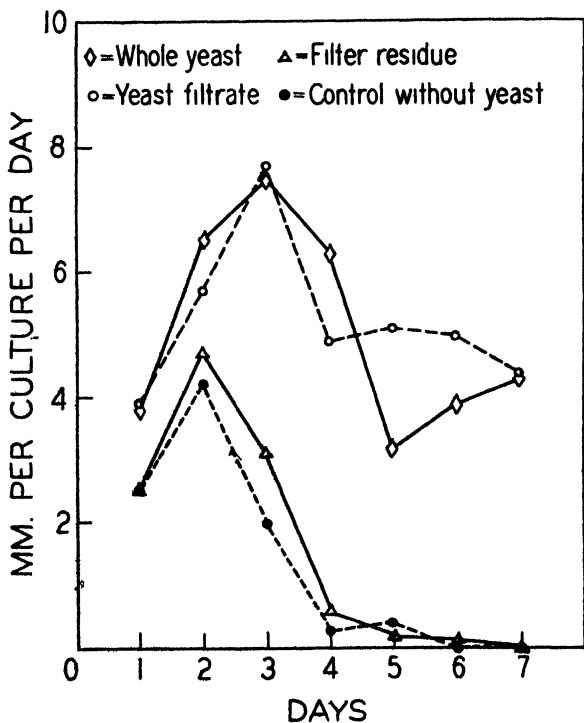


FIG. 4. Curves showing effects of fractions of yeast separated by filtration.

1 liter of water, filtered, the residue re-extracted in like manner twice more, the extracts mixed, and equivalent aliquots of extract, of residue, and of whole yeast resuspended in nutrient and tested. The results are shown in figure 4, and indicate that water extraction does remove all of the effective material. Like results were obtained when centrifugation was substituted for filtration. Aliquots dried *in vacuo* and weighed showed that the water-soluble material made up 36 per cent. of the total dry weight of the yeast. The residual 64 per cent. was inert as regards growth-promoting properties.

### DIFFUSIBILITY

A water extract of yeast was subjected to dialysis through a cellophane membrane. A stirring device was used to insure thorough mixing. The extract was placed within a tubular membrane which was immersed in a quantity of water approximately equal to that within the membrane. The external water was renewed every hour for 6 consecutive periods, the 6 extracts being finally mixed. Assuming complete equilibrium between external and internal solution at the end of each period, the amount of dialyzable material left within the membrane at the end of this treatment should be about 1.5 per cent. of that originally present, 98.5 per cent. having passed into the diffusate. The growth-promoting properties of the two fractions are shown by the data presented in figures 5 and 6. Most of the effective material passed through the membrane. The small residual activity of the non-diffusible fraction was probably due to incomplete dialysis. The diffusible material represented 23 per cent. of the weight of whole yeast. At least 77 per cent. of the yeast was thus shown to be inert.

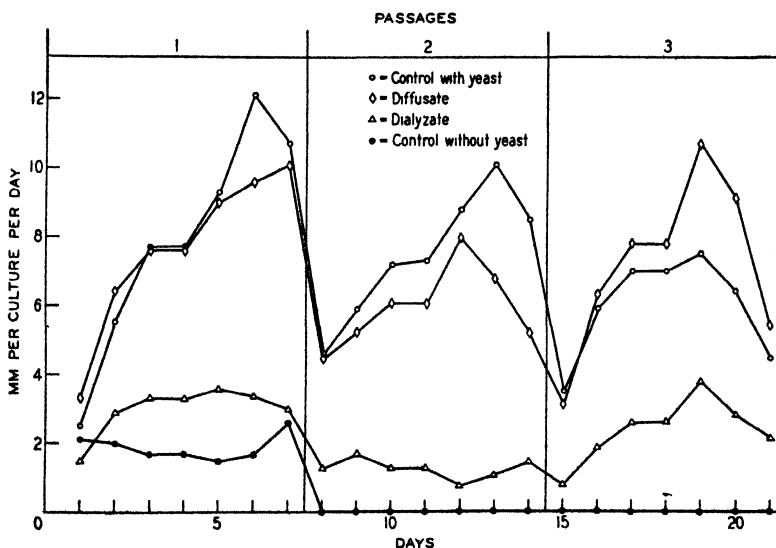
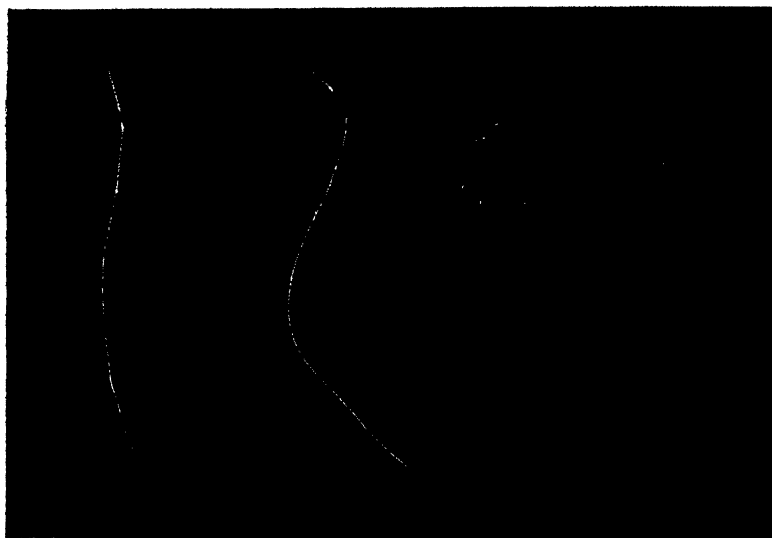


FIG. 5. Curves showing effects of yeast fractions separated by dialysis.



*Photograph by J. A. CARLILE*

FIG. 6. Effects of fractions of yeast separated by dialysis. Reading from left to right: A, control with whole yeast extract. B, fraction passing through cellophane. C, fraction retained by cellophane. D, control without accessory materials. Photograph taken at the end of the 3rd passage.  $\times 0.6$ .

#### SOLUBILITY IN 85 PER CENT. ALCOHOL

An aliquot of a water extract of yeast was reduced to dryness over a steam bath and then extracted 3 times with boiling 85 per cent. ethyl alcohol, refluxing for  $\frac{1}{2}$  hour with each extraction. The extracts were mixed, both fractions dried, the dry residues resuspended in water and tested. The results are shown in figure 7, and demonstrate that all of the growth-promoting material was soluble in 85 per cent. alcohol. This fraction represents 18 per cent. of the total weight of dry yeast.

#### SOLUBILITY IN 100 PER CENT. ALCOHOL

An 85 per cent. alcohol extract of yeast was dried and then extracted 3 times with portions of absolute ethyl alcohol freshly distilled over CaO to insure complete dryness. The fractions were dried, redissolved in water and tested, both separately and in combination. A small portion of the 100 per cent. alcohol-soluble material prepared in this way failed to redissolve. This insoluble material was dark brown, highly aromatic, and oily. It proved to be soluble in ether, slightly soluble in alcohol, and insoluble in water. It appears to be lipoidal in nature, and was presumably carried over in an emulsified form in the previous extraction. The fractions soluble in water but separated by extraction with absolute alcohol were tested separately and

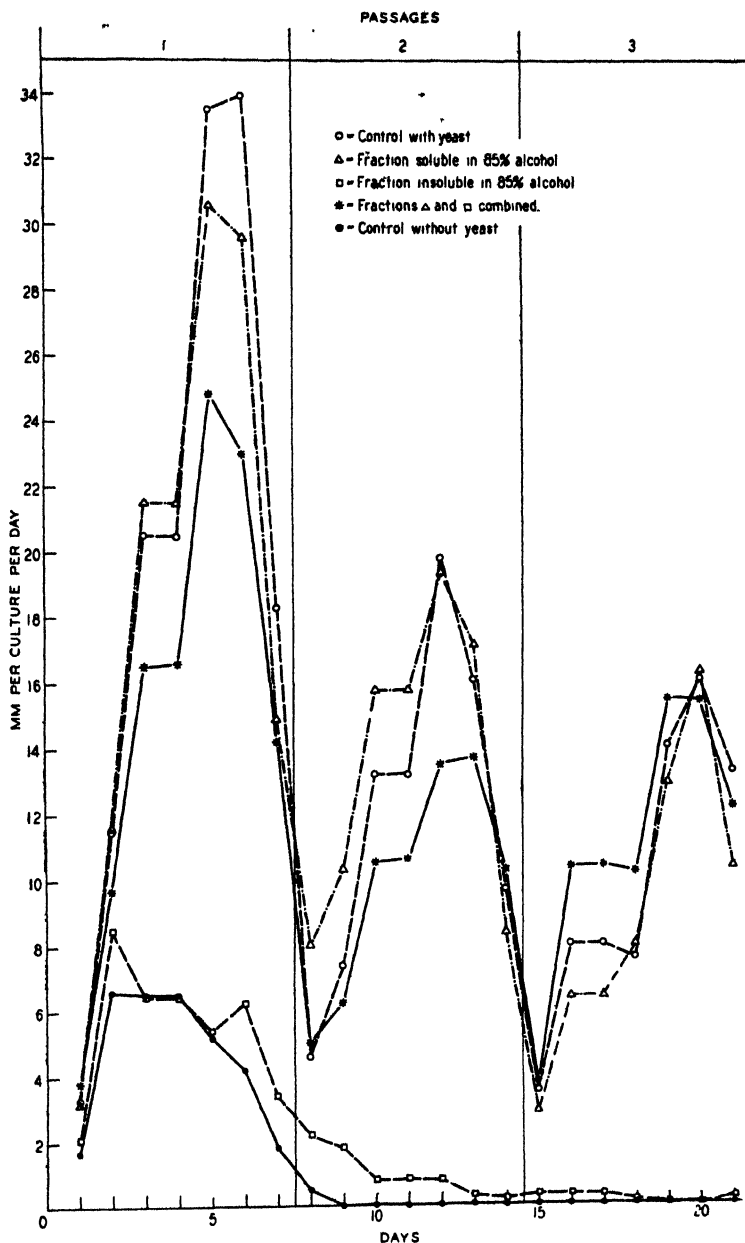


FIG. 7. Effect of yeast fractions separated on the basis of solubility in 85 per cent. alcohol. The differences between growth rates in different passages are the result of uncontrolled temperature and are to be ignored. The growth rate in the 1st passage was extraordinarily high, the 2nd and 3rd passages being more nearly average.



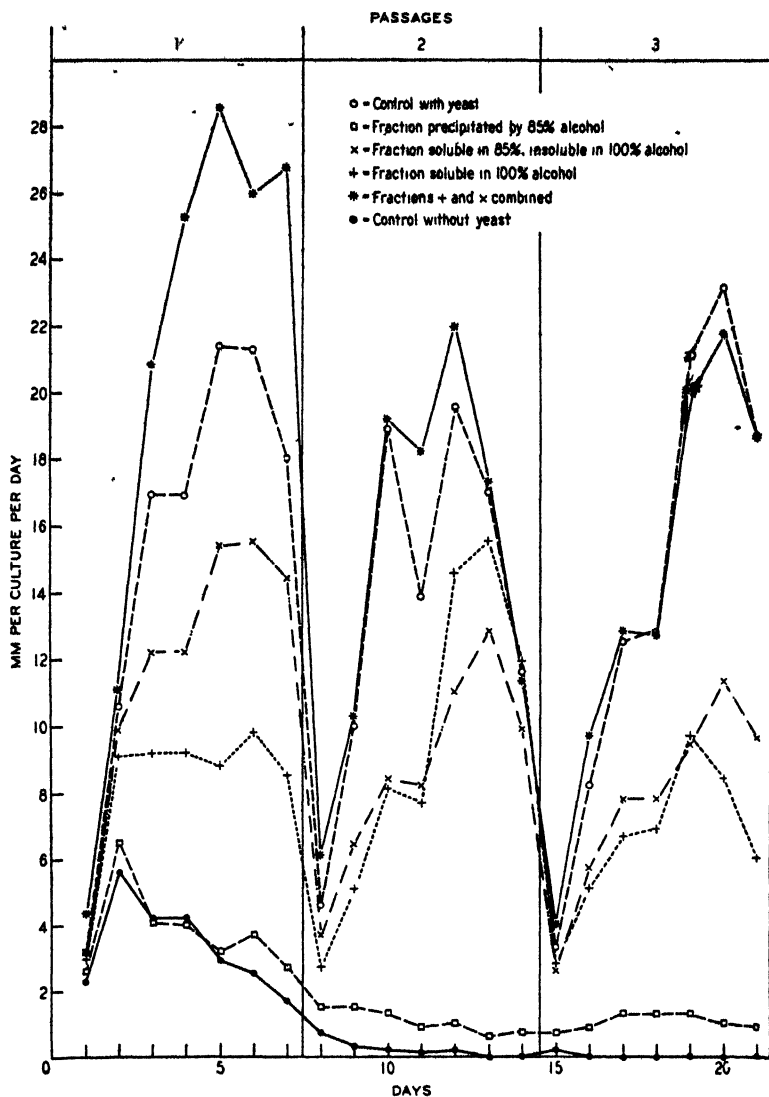
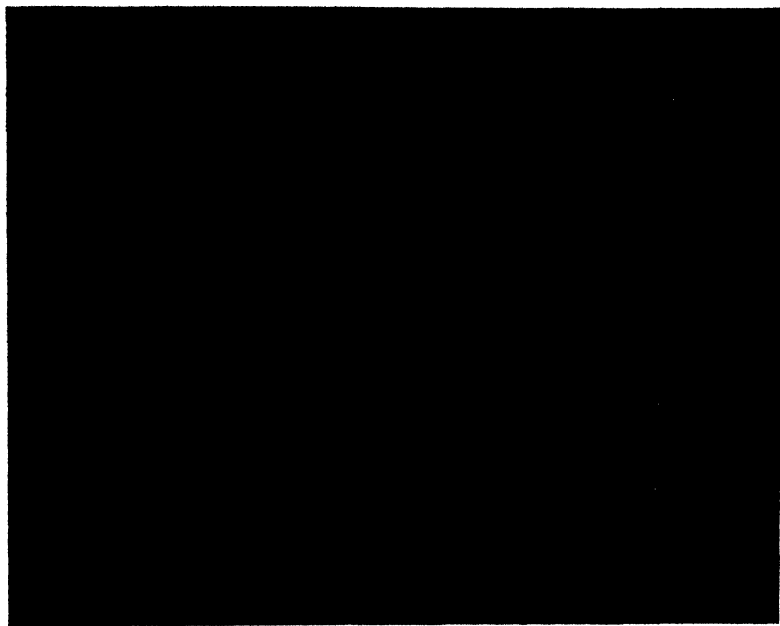


FIG. 8. Effects of yeast fractions separated by extraction with 85 and 100 per cent. alcohol.

in combination. As will be seen from figures 8 and 9, this treatment separates the yeast material into two portions, either of which will maintain growth continuously at a low level. Both are necessary for satisfactory growth. This is the first indication obtained that more than one accessory substance or group of substances obtainable from yeast are required by tomato roots. The material soluble in 85 per cent. alcohol but insoluble in



*Photograph by J. A. CARLILE*

FIG. 9. Effects of yeast fractions separated on the basis of solubility in 85 and 100 per cent. alcohol. Reading from left to right: A, control in whole yeast extract. B, control without accessory material. C, material precipitated by 85 per cent. alcohol. D, material soluble in 85 per cent. alcohol, insoluble in 100 per cent. alcohol. E, material soluble in 100 per cent. alcohol. F, fractions D and E mixed. Photographs taken at the end of 3 passages.  $\times 0.5$ .

100 per cent. alcohol represented 13 per cent. of the total weight of yeast, that soluble in 100 per cent. alcohol, 5 per cent.

#### SOLUBILITY IN ETHER

An 85 per cent. alcohol extract of yeast was dried and then extracted 3 times with ethyl ether. Figure 10 shows the results of testing the fractions separated in this manner. The effective material was insoluble in ether and therefore not lipoidal. The ether-soluble fraction prepared in this way represented about 0.7 per cent. of the weight of dry yeast.

#### STABILITY IN TRICHLOROACETIC ACID

An 85 per cent. alcohol extract of yeast was dried, a 40 per cent. solution of trichloroacetic acid added, the mixture boiled until the odors of acetic acid and of chlorine were no longer detectable, and then cooled and adjusted to the original pH (about 5.5). No precipitate occurred during this treatment, and on testing, practically all of the activity was recoverable in the material so treated (fig. 11). The active material is, therefore, probably not

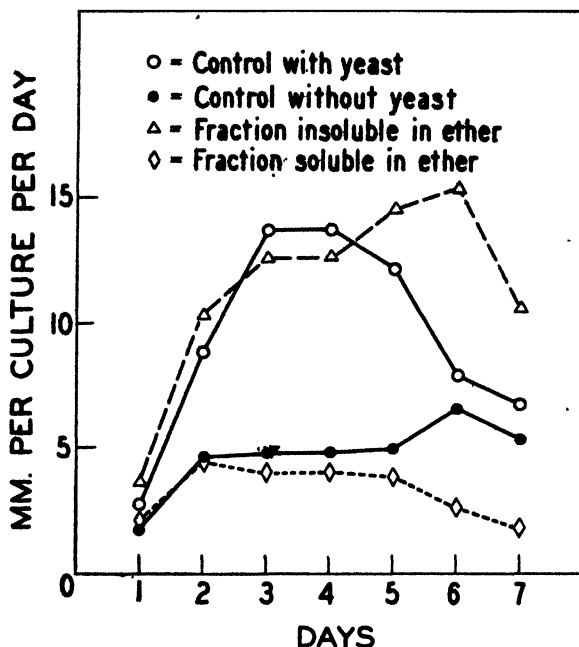


FIG. 10. Effect of yeast fractions separated on the basis of ether solubilities.

proteinaceous in nature, but such treatment does not eliminate the possibility of its being proteose.

TABLE I  
SUMMARY OF RESULTS

TREATMENT	WEIGHT AS PERCENTAGE OF WEIGHT OF WHOLE YEAST		GROWTH-PROMOTING EFFECT OF MATERIAL TREATED
	INSOLUBLE	SOLUBLE	
Water .....	64	—	—
	—	36	+++
85 per cent. alcohol .....	16	—	—
	(2)*	18*	+++
100 per cent. alcohol .....	13	—	+
	—	5	+
Ether .....	4.6	—	+++
	—	0.4	—
Trichloroacetic acid .....	—	—	+++
Nitrous oxide .....	—	—	—

\* About 2 per cent. of this fraction was lost in the process of preparation due to volatilization and other unavoidable causes. This has been placed in the "insoluble" column so as to account for all the weight.

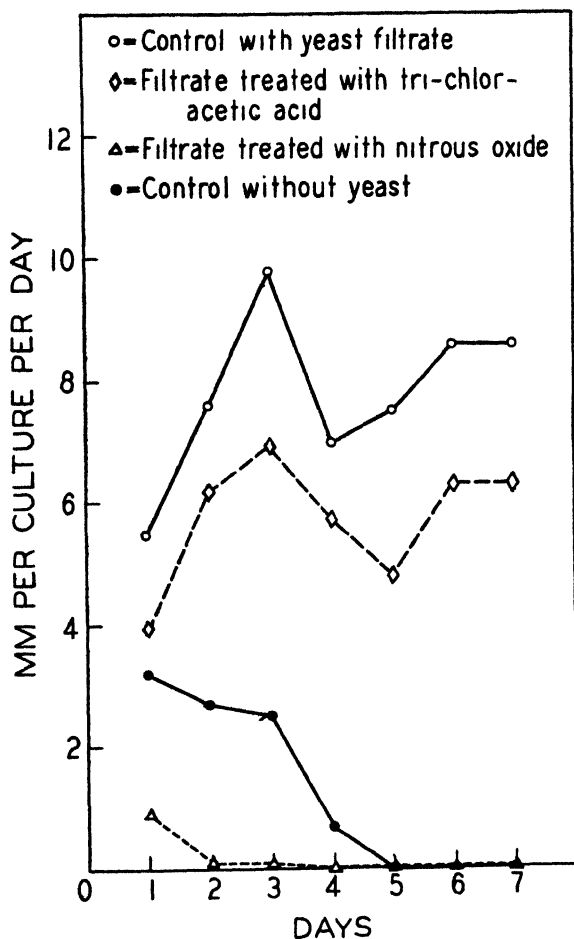


FIG. 11. Effects of treatment of yeast extract with boiling trichloroacetic acid and with nitrous oxide.

#### STABILITY IN NITROUS OXIDE

An aliquot of yeast extract was shaken for  $\frac{1}{2}$  hour in an atmosphere of  $\text{NO}_2$  at pH 3, the pH readjusted to 5.5, and the growth-promoting properties tested. As shown in figure 11, this treatment completely destroyed the effectiveness of the extract. This suggested the probable importance of amino-groups.

The results of this analysis are summarized in table I.

#### Conclusions

From the results of this preliminary fractionation it is clear that, since the yeast material which exerts an essential growth-promoting effect on iso-

lated tomato roots is insoluble in ether, it cannot be lipoidal in nature, nor can it be one of the generally recognized growth "hormones" such as "auxin" or "hetero-auxin." It is equally clear that, being soluble in 85 per cent. alcohol, stable in trichloroacetic acid, and stable to repeated boiling, it cannot be any protein unless possibly prolamine. On the other hand, its destruction by nitrous oxide suggests that the amino groupings may play an important rôle in bringing about its growth-promoting effects. The material soluble in absolute alcohol, which is essential for satisfactory growth, cannot contain amino-acids other than small amounts of prolines, but the other effective fraction, insoluble in 100 per cent. but soluble in 85 per cent. alcohol, must contain considerable amounts of amino acids. A tentative analysis has shown amino acids to represent 20 to 30 per cent. of this fraction. They constitute a possible source of the growth-promoting properties of the absolute alcohol-insoluble fraction. Since this fraction makes up only 13 per cent. of the weight of whole yeast and is itself about 25 per cent. amino acids, effective amino acid materials cannot, in the yeast extract, exceed a concentration of about 4 mg. per liter of nutrient. The fraction soluble in absolute alcohol is likewise effective at a concentration of 4-5 mg. per liter of nutrient. Further analysis will be discussed elsewhere.

### Summary

Material obtainable from yeast has been shown to be essential for satisfactory growth *in vitro* of excised tomato roots. Optimal results were obtained with about 100 mg. of yeast per liter of nutrient. Treatment of yeast with various reagents showed all of the effective material to be soluble in H<sub>2</sub>O and 85 per cent. ethyl alcohol, stable in trichloroacetic acid, unstable in nitrous oxide, and insoluble in ethyl ether. At least 82 per cent. of the yeast material is inert, only 18 mg. of an 85 per cent. alcohol extract per liter of nutrient being needed for optimal results. Extraction of this material with 100 per cent. alcohol separates it into two fractions, both of which are essential for satisfactory growth. The material insoluble in 100 per cent. alcohol contains considerable quantities of amino acids, and it is suggested that these may play an important rôle as growth-promoting substances for isolated tomato roots.

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# AMINO ACIDS IN THE NUTRITION OF EXCISED TOMATO ROOTS

PHILIP R. WHITE

(WITH FIVE FIGURES)

## Introduction

A preliminary study of the growth-promoting materials obtainable from yeast and essential for the nutrition of excised tomato roots (10) has shown that all of these materials are soluble in 85 per cent. ethyl alcohol, insoluble in ether, and stable in boiling trichloroacetic acid. They can be further divided into two fractions by extracting with absolute alcohol. Both fractions are essential for satisfactory growth. The present paper deals with results of an analysis of that fraction which is soluble in 85 per cent. alcohol and insoluble in 100 per cent. alcohol and ether. The basic nutrients, cultural methods, and materials used have been the same as those previously described.

As was stated in the earlier report, the material soluble in 85 per cent. alcohol contains, in addition to many unknown substances, a very considerable amount of free amino acid. OSBORNE and WAKEMAN (7) give the total N content of a similarly prepared fraction, soluble in 80 per cent. alcohol, as 6.5 per cent. of the total yeast N or 12 per cent. of the weight of the fraction. They do not report the amino nitrogen content. About 3.5 per cent. of the total weight of this fraction, or 29 per cent. of its total N, has been found by the present writer to be amino nitrogen (van Slyke method). Between 20 per cent. and 25 per cent. of the fraction is, therefore, amino acid. As was suggested in the earlier paper, this high amino acid content obviously suggests that this constituent may play an important part in bringing about the growth-promoting activity of the fraction. As a check on this possibility an aqueous extract of yeast was shaken for one-half hour in an atmosphere of  $\text{NO}_2$  at pH 3.0, adjusted to pH 5.6, and its growth-promoting properties tested. The effectiveness was found to be completely destroyed. One of the primary effects of this treatment is deaminization, although such treatment is too drastic to furnish an unequivocal diagnosis. This observation supports the presumption that the amino acids might be important factors in producing the effectiveness of the fraction. It seemed desirable, therefore, to determine in some detail the effects of commonly occurring amino acids on growth of these roots.

## Experimentation

### EFFECTS OF KNOWN AMINO ACIDS ON GROWTH

In a preliminary survey, 19 nutrients were made up, each one containing the usual salts and carbohydrate but with a single amino acid taking the



place of accessory organic material. Amino acids were obtained from two sources, as follows: From Dr. C. S. MARVEL, University of Illinois, l-cystine, l-tyrosine, dl-valine, dl-isoleucine, dl-norleucine, dl-methionine, dl-phenyl-alanine, dl-lysine-2HCl. From Pfanstiehl Chemical Co., Waukegan, Illinois, glycine, d-arginine, l-leucine, l-tryptophane, l-proline, l-hydroxyproline, l-histidine-HCl, l-aspartic acid, d-glutamic acid, dl-serine, dl-alanine. Hydroxy-glutamic acid, citrulline, and ROSE's  $\alpha$ -amino- $\beta$ -hydroxy-n-butyric acid (1) were not available for study.

A concentration of 5 mg. of amino acid per liter of nutrient (5 p.p.m.) was first tested. This was slightly less than the total weight of amino acids (8 mg.) shown by MEISENHIMER to be present (2) in what has been determined to be the optimal concentration of yeast (10). The results are shown in figure 1. At a concentration of 5 mg. per liter, 7 of the 19 amino acids

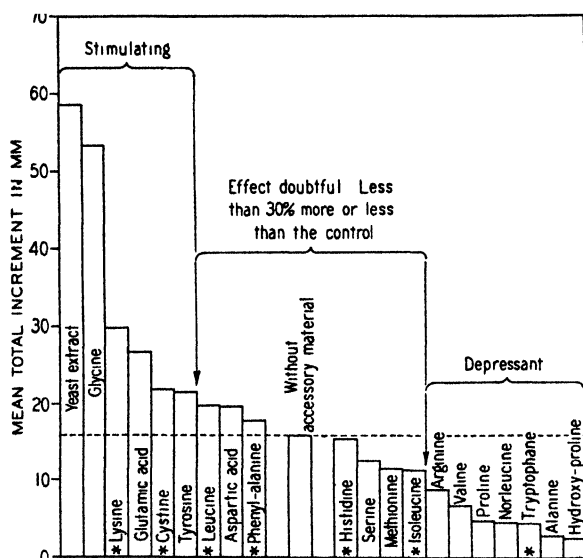


FIG. 1. Relative effectiveness of single amino-acids when tested at a concentration of 5 mg. per liter, in the absence of all other accessory materials. Those marked with an asterisk are considered by ROSE (9) to be essential for growth of rats.

studied proved to be depressant, and only 3—glycine, lysine, and glutamic acid—were definitely stimulating. Under these conditions glycine gave results almost equal to the control.

Optimal concentrations were then determined individually for each acid. For this purpose, each was tested at concentrations of 50, 15, 5, 1.5, 0.5, 0.15, and 0 mg. per liter of nutrient. Isoleucine, norleucine, methionine, serine, and arginine, which gave regularly increasing growth rates with decreasing concentration throughout this range, with the optimal growth better than the controls, were tested at additional concentrations of 0.05 and 0.015 mg. per

TABLE I

GROWTH INDICES OF ISOLATED TOMATO ROOT-TIPS AT VARIOUS CONCENTRATIONS OF SINGLE AMINO ACIDS. THE GROWTH RATE IN THE ABSENCE OF ALL ACCESSORY MATERIAL IS TAKEN AS 100

AMINO ACID	CONCENTRATION—MG. PER LITER								
	50	15	5	1.5	0.5	0.15	0.05	0.015	0
Glutamic acid .....	35	133	97	102	97	98	.....	.....	100
Aspartic acid .....	15	124	109	98	106	86	.....	.....	100
Glycine .....	38	84	306	146	97	104	.....	.....	100
Lysine .....	31	157	216	194	200	171	.....	.....	100
Phenyl-alanine .....	52	86	118	98	100	95	.....	.....	100
Proline .....	30	41	56	134	93	74	.....	.....	100
Cystine .....	70	70	100	128	104	100	.....	.....	100
Hydroxyproline .....	7	9	18	71	127	99	.....	.....	100
Leucine .....	20	50	110	114	118	103	.....	.....	100
Histidine .....	16	51	62	92	109	100	.....	.....	100
Valine .....	5	22	44	104	108	97	.....	.....	100
Methionine .....	34	42	48	67	100	139	100	.....	100
Serine .....	11	33	66	82	107	111	93	98	100
Norleucine .....	10	15	22	32	56	128	124	103	100
Arginine .....	11	26	74	110	110	127	100	.....	100
Tyrosine .....	19	42	76	99	84	103	.....	.....	100
Isoleucine .....	21	31	51	81	124	138	174	99	100
Tryptophane .....	13	20	36	78	85	86	.....	.....	100
Alanine .....	6	10	14	24	90	76	.....	.....	100

liter. This series included about 3000 individual cultures so that it was necessary to run them in 10 consecutive sets, each with a separate set of controls. In order to facilitate comparison of the sets within this series with one another, the results have been expressed as percentages of the controls. The results are shown in table I. All amino acids studied proved to be depressant at 50 mg. per liter (50 p.p.m.). Alanine, valine, hydroxyproline, and norleucine were quite definitely so. Tryptophane and alanine were depressant at all concentrations studied. Tyrosine was apparently not beneficial at any concentration tested. Valine, histidine, serine, phenyl-alanine, methionine, cystine, leucine, norleucine, arginine, aspartic acid, glutamic acid, proline, and hydroxyproline showed slight stimulation at some point in the concentration range investigated. Glycine, lysine, and isoleucine were markedly stimulating at favorable concentrations. The optimal concentrations varied from 15 p.p.m. in the case of glutamic and aspartic acids down to 0.05 p.p.m. in the case of isoleucine. A typical series of results is shown in figure 2 (glycine).

From these data an amino acid mixture having the provisional constitution shown in the first column of table II was chosen for study. MEISEN-

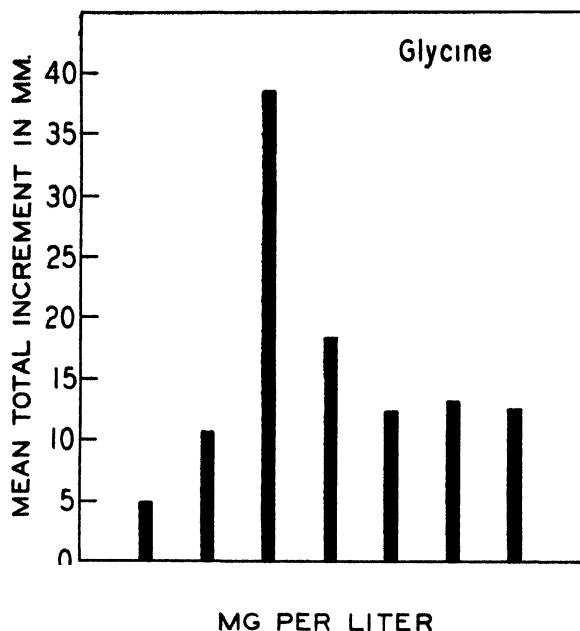


FIG. 2. Histogram showing total increments obtained in a week's time in nutrients containing various concentrations of glycine, without other accessory material.

HEIMER has studied in detail the nitrogenous constituents of hydrolyzed yeast (2, 3). Although his figures are much higher than would be the case for an alcohol extract, it is clear that such an extract could never contain *more* of any amino acid than he has indicated. His figures have, therefore, been recalculated to give comparable units and are presented for comparison.

The last column of the table is of especial interest. In most cases the experimentally determined optimum concentration for nutrition of isolated tomato root-tips did not differ widely from the amounts actually found in a hydrolyzed yeast extract. But the optimal amounts found for glycine, cystine, glutamic acid, and aspartic acid were so much higher than can possibly be present in yeast (MEISENHEIMER, see table II) that some explanation would seem necessary. It is to be noted that three of these—glycine, glutamic acid, and aspartic acid—are not among the nine amino acids recognized by ROSE (8, 9) as essential for animals. It seemed possible that in a complete mixture these three might prove to be partially or wholly interchangeable with some of the other amino acids. However, it is to be remembered that MUELLER (5) found diphtheria bacilli to require one of these, glutamic acid, in *unexpectedly* large amounts.

On the basis of the information contained in the data presented above, an amino acid mixture was prepared containing 15 mg. each of glutamic acid

TABLE II  
CONCENTRATIONS OF SINGLE AMINO ACIDS OPTIMAL FOR GROWTH OF ISOLATED  
TOMATO ROOT-TIPS

AMINO ACID	OPTIMUM CONCENTRATION (a)	TOTAL CONCENTRATION IN OPTIMAL YEAST CONTENT (FROM MEISENHEIMER) (b)	RATIO a/b
	<i>mg./liter</i>		
Glycine .....	5.000	0.03	166.7
Cystine .....	1.500	0.04	37.5
Lysine .....	5.000	0.52	9.6
Glutamic acid .....	15.000	0.62	24.1
Isoleucine .....	0.050	0.23	0.2
Norleucine .....	0.150	.....	.....
Methionine .....	0.150	0.14*	1.1
Serine .....	0.150	1.14	0.1
Proline .....	1.500	0.16	0.9
Hydroxyproline .....	0.500	0.38	1.3
Phenyl-alanine .....	5.000	1.13	4.4
Leucine .....	0.500	0.70	0.7
Valine .....	0.500	0.84	0.6
Arginine .....	0.150	0.31	0.5
Aspartic acid .....	15.000	0.38	39.3
Histidine .....	0.500	0.24	2.1
Total .....	50.650	6.86	7.4

\* MEISENHEIMER (2, 3) does not mention methionine by name but speaks of "sulfur containing amino acids other than cystine" as being present in this amount. It is assumed that the "amino acid" to which he refers was probably methionine.

and aspartic acid, 5 mg. of glycine, lysine, and phenyl-alanine, 1.5 mg. of cystine and proline, 0.5 mg. of hydroxyproline, leucine, histidine, and valine, 0.15 mg. of methionine, serine, norleucine, and arginine, and 0.05 mg. of isoleucine, totaling 50.65 mg. of amino acid in each liter of solution. Since this was about 7 times the total amount of amino acid contained in the optimal concentration of yeast (MEISENHEIMER, 2) and since concentrations, both relative and absolute, have repeatedly proved of great importance, the mixture was tested at 1, 0.3, 0.1, 0.03, and 0.01 times this concentration. The results are shown in figure 3. As was the case with single amino acids, a concentration of 50 mg. per liter was quite depressant, the optimum lying at about 15 mg. per liter, one-third the concentration originally chosen.

As has already been suggested, the importance of many of the amino acids used above, especially in the presence of the other amino acids, was subject to considerable doubt, since it is known that in animal nutrition cer-

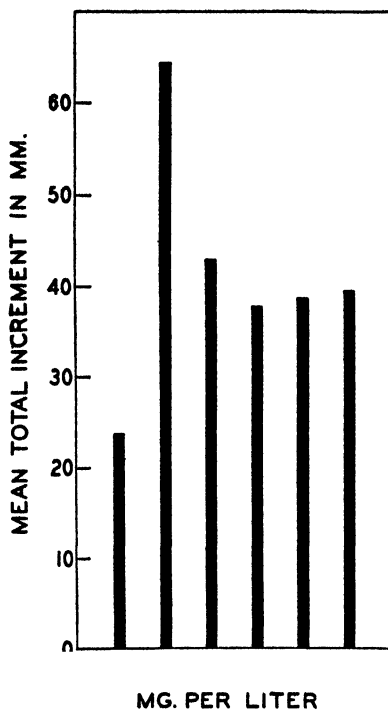


FIG. 3. Effects on growth of various concentrations of a mixture containing 16 amino acids (see text).

tain acids may substitute for one another (ROSE, *et al.*). It thus seemed probable that some of these amino acids, which when taken singly proved to be beneficial, might nevertheless be unnecessary for optimal growth. To test this, a series of cultures was made, in each of which one amino acid was employed at 10, 1, 0.1, and 0 times the concentration (one-third that shown in table II) found to be optimal in the last experiment, while all other amino acids were present at the optimal concentrations. This test involved 64 combinations, 1280 cultures. In each set of cultures a solution containing all amino acids except the one under examination was used as control. The results, given as percentages of these controls are presented in table III.

It will be seen from the table that of the 16 amino acids which have a stimulating effect at some concentration when taken alone, 7 fail to exert such an effect when in the presence of all the others. These 7 are glycine, aspartic acid, hydroxyproline, cystine, methionine, arginine, and norleucine. Reference to table II will show that of these, 3—glycine, cystine and aspartic acid—were among those which, when taken singly, gave optimum results at concentrations greater than those existing in the optimal concentration of yeast. It seems probable that the originally observed effects of these 3 (table

TABLE III

GROWTH INDICES OF ISOLATED TOMATO ROOT-TIPS GROWN IN A COMPLETE AMINO ACID MIXTURE (TABLE II) IN WHICH THE CONCENTRATIONS OF SINGLE AMINO ACIDS WERE VARIED. THE GROWTH RATE IN A MIXTURE, COMPLETE EXCEPT FOR THE AMINO ACID UNDER EXAMINATION, IS TAKEN AS 100

AMINO ACID	RELATIVE CONCENTRATION OF THE AMINO ACID WHOSE CONCENTRATION IS BEING VARIED. ONE-THIRD OF THE VALUE INDICATED IN TABLE II AS INDIVIDUALLY OPTIMAL IS TAKEN AS 1 (COLUMN 2) (SEE TEXT)			
	10	1.0	0.1	0
Histidine .....	136	115	108	100
Proline .....	85	120	102	100
Serine .....	88	122	104	100
Valine .....	82	124	114	100
Phenyl-alanine .....	48	124	124	100
Glutamic acid .....	35	144	138	100
Lysine .....	56	162	159	100
Leucine .....	80	96	133	100
Isoleucine .....	80	99	118	100
Arginine .....	100	104	102	100
Methionine .....	68	98	96	100
Cystine .....	60	89	90	100
Aspartic acid .....	14	72	97	100
Glycine .....	53	67	89	100
Hydroxyproline .....	63	80	92	100
Norleucine .....	61	82	77	100

II) may have been due to traces of other essential amino acids present as impurities in the samples studied, or else that they may function only as building blocks for other essential acids present in the complete mixture. A fourth amino acid, methionine, discarded by this process, is known to be a common contaminant in commercial preparations of leucine (MUELLER, 6) and was possibly supplied in the requisite amount in this material. Of the 4 acids giving aberrant results (table II), only glutamic acid remains as apparently essential *sui generis*. Using diphtheria bacilli, MUELLER likewise found the optimal concentration of glutamic acid to be abnormally high.

If the acids found to be essential for rats (ROSE, 9), for diphtheria bacilli (MUELLER, 5), and for tomato roots (this work) are tabulated, the very close correspondence between the three sets is evident. Only two amino acids—proline and serine—present in the list developed for tomato root-tips are not present in either ROSE's or MUELLER's lists. Four acids—cystine, methionine, glycine, and tryptophane—are to be found in one or both of these lists but not in the present one. Tryptophane alone, of those found essential by both ROSE and MUELLER, has under no circumstances appeared.

beneficial for tomato root-tips. In view of its close chemical relationship to heteroauxin, which is known to have a depressant effect on root growth, tryptophane may have a place which is unique. Further study will be required before its true position can be considered established.

TABLE IV

AMINO ACIDS ESSENTIAL FOR OR BENEFICIAL TO ORGANISMS OF THREE WIDELY SEPARATED GROUPS

RATS (ROSE)	TOMATO ROOTS (WHITE)	DIPHTHERIA BACILLI (MUELLER)
Histidine	Histidine	Histidine
Phenyl-alanine	Phenyl-alanine	Phenyl-alanine
Lysine	Lysine	.....
Leucine	Leucine	.....
Isoleucine	Isoleucine	.....
Cystine	.....	Cystine
Tryptophane	.....	Tryptophane
Threonine*	(Not tested)	(Not tested)
.....	Valine	Valine
.....	Glutamic acid	Glutamic acid
.....	Proline	.....
.....	Serine	.....
.....	.....	Glycine
.....	.....	Methionine

\* =  $\alpha$ -amino- $\beta$ -hydroxy-n-butyric acid (4).

A provisional amino acid mixture which appeared to be complete and possibly optimal then contained: 5 mg. of glutamic acid, 1.5 mg. each of phenyl-alanine, lysine, and histidine, 0.5 mg. of proline, 0.15 mg. of valine, 0.05 mg. of serine, 0.015 mg. of leucine, and 0.0015 mg. of isoleucine per liter of nutrient. The total amino acid content of this mixture was 10.2165 mg. per liter.

Since such an amino acid mixture was developed in the hope of replacing therewith the fraction of yeast soluble in 85 per cent. ethyl alcohol but insoluble in 100 per cent. alcohol, a series of cultures was carried out using this mixture together with a 100 per cent. alcohol extract of yeast in amount equivalent to the standard amount of whole yeast regularly used as control. The results are shown in figures 4 and 5. Such a mixture of amino acids, when added to the yeast material soluble in absolute alcohol, is adequate for the maintenance of continuous growth for at least three passages, at a level equal to or nearly equal to that obtained with whole yeast. The entire effect of the yeast material soluble in 85 per cent. alcohol but insoluble in absolute alcohol, insofar as it can be observed in this way, is then to be attributed to its amino acid content.

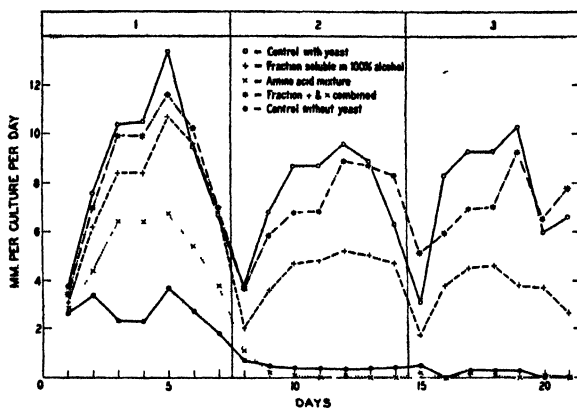


FIG. 4. Graph showing the effects during three passages<sup>1</sup> of a mixture of 16 amino acids (see text) when taken alone and in the presence of a 100 per cent. alcohol extract of yeast.

A B C D E



(Photograph by J. A. CARLILE)

FIG. 5. Reading from left to right: A, control with yeast extract. B, control without accessory material. C, amino acid mixture alone. D, yeast material soluble in 100 per cent. alcohol, insoluble in ether. E, same as D but with amino acid mixture (C) added. Photograph taken at the end of the third passage.  $\times 1$ .

There remains to be identified only the yeast material soluble in absolute alcohol, representing 0.0005 per cent. of the total nutrient.

### Summary and conclusions

In an earlier paper it was shown that yeast extract contains certain materials essential for growth of isolated tomato roots. These are all soluble

<sup>1</sup> "Passage 1" in this experiment is passage 196, counting from the time of isolation, the roots having been maintained through 195 passages under standard conditions before being used in this experiment.



in 85 per cent. ethyl alcohol, and insoluble in ether. Further extraction with 100 per cent. alcohol segregates two fractions, both of which are necessary for satisfactory growth. Data have been presented in this paper which show that the fraction which is soluble in 85 per cent. alcohol, but insoluble in 100 per cent. alcohol and ether, may be replaced by a mixture of 9 amino acids without appreciably reducing the growth rate. The amino acid content of this fraction appears to be entirely responsible for its growth-promoting effect, at least so far as the requirements of isolated tomato root-tips are concerned. Such a mixture contains the following: glutamic acid, lysine, histidine, phenyl-alanine, leucine, isoleucine, valine, serine, and proline. All other amino acids appear to be unessential under the conditions of the experiment reported. The fraction soluble in 100 per cent. alcohol remains to be identified.

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# VITAMIN B<sub>1</sub> IN THE NUTRITION OF EXCISED TOMATO ROOTS

PHILIP R. WHITE

(WITH FIVE FIGURES)

## Introduction

In previous studies of the growth-promoting materials obtainable from yeast and essential for the nutrition of excised tomato roots, these materials were shown to be soluble in 85 per cent. ethyl alcohol and insoluble in ether. Further extraction with absolute alcohol segregated two fractions both of which proved essential for optimal growth (5). The effectiveness of the insoluble residue left after absolute alcohol extraction of the 85 per cent. alcohol-soluble material was shown to be largely if not entirely attributable to the amino acids contained therein (6). The material soluble in absolute alcohol was not studied.

Besides the properties mentioned above, this material was shown to be stable to prolonged boiling in trichloroacetic acid, resistant to repeated drying, and resistant to moderately prolonged autoclaving such as was regularly employed in the preparation of nutrients (4). Further tests showed that its activity was completely destroyed by boiling for 2 hours in 1 N NaOH. Boiling for a similar period in 6 N HCl gave a product which supported apparently normal growth during the time necessary for a single passage (one week) but not for longer periods. The writer wishes to thank Dr. HUBERT S. LORING for carrying out these last treatments and for valuable advice during the conduct of the work.

The properties of this material as now known (solubility in 100 per cent. alcohol, destruction by alkali, and moderate stability in acid) are characteristic of vitamin B<sub>1</sub>. It thus appeared possible that vitamin B<sub>1</sub> might be an important constituent of the fraction in question. Nevertheless, certain unpublished evidence obtained early in the conduct of this investigation seemed to oppose this view. Before any attempt was made to analyze the yeast material, a number of substances reported to act as growth stimulators was studied. These included inositol, vitamin B<sub>1</sub>, heteroauxin,  $\beta$ -indolyl-propionic acid, haemin, yeast nucleic acid, glutathione, and a number of different sugars. None of these substances gave any evidence of stimulating growth of isolated tomato roots when tested alone. The effort to approach the problem in this way was therefore abandoned.

Nevertheless, out of the work already published there has grown the conviction that failure to demonstrate the effectiveness of any material when studied alone cannot be taken as conclusive evidence of its ineffectiveness in a complete nutrient. It seemed possible, therefore, that vitamin B<sub>1</sub>,

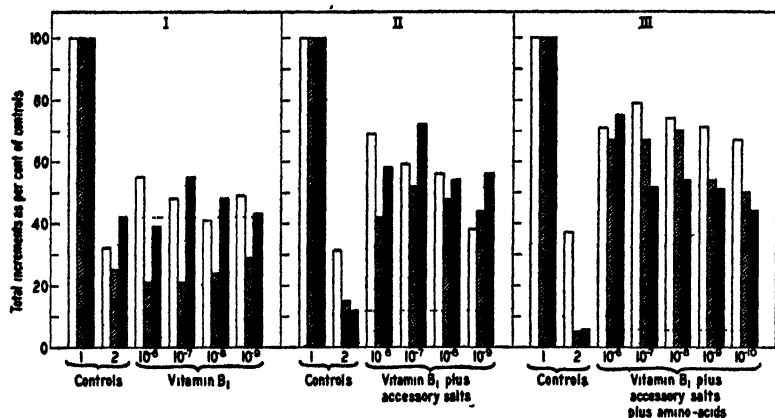


FIG. 1. Histograms showing growth of isolated tomato roots in nutrients containing standard salts, and sugar, with the addition in I of vitamin  $B_1$  at concentrations of  $10^{-6}$  to  $10^{-9}$ , of vitamin  $B_1$  and accessory salts in II, and of these materials and amino acids in III. The open columns represent the first week's results (average of 20 cultures in each nutrient), the hatched columns the second week, and the blocked-in columns the third week. The transverse dotted line in each case is placed at the level of the negative control (without accessory organic material) in the third passage. All experimental increments must be above the level of this line to indicate growth stimulation. Control 1 is with yeast extract added to the basic nutrient, control 2 without accessory organic material of any sort.

although shown to be ineffective when taken alone, in the presence of the amino acid mixture developed in recently reported work (6) might prove to be of importance.

A study of the effects of vitamin  $B_1$  in combination with these amino acids was, therefore, undertaken. While this work was under way the notes of BONNER (1) and of ROBBINS and BARTLEY (2) appeared, reporting evidence of the importance of vitamin  $B_1$  in the nutrition of isolated roots. The present work is, therefore, a supplement to the work of these authors.

The basic nutrients, cultural methods, and materials used were the same as those previously described (5, 6). At the time these experiments were begun the roots were in the 196th passage.

### Experimentation

Cultures were first carried out in the nutrient heretofore designated as basic, containing standard salts (including iron), accessory salts, and sugar (5), alone and with the following additions: amino acids only; vitamin  $B_1$  only; amino acids and vitamin  $B_1$  together; and yeast extract as control.

<sup>1</sup> This notation has been employed in the figures to conserve space. A concentration of  $10^{-6}$  parts of vitamin  $B_1$  in 1 part of nutrient is equivalent to 1 mg. per liter or 1 ppm. or 1  $\gamma$  per cc.

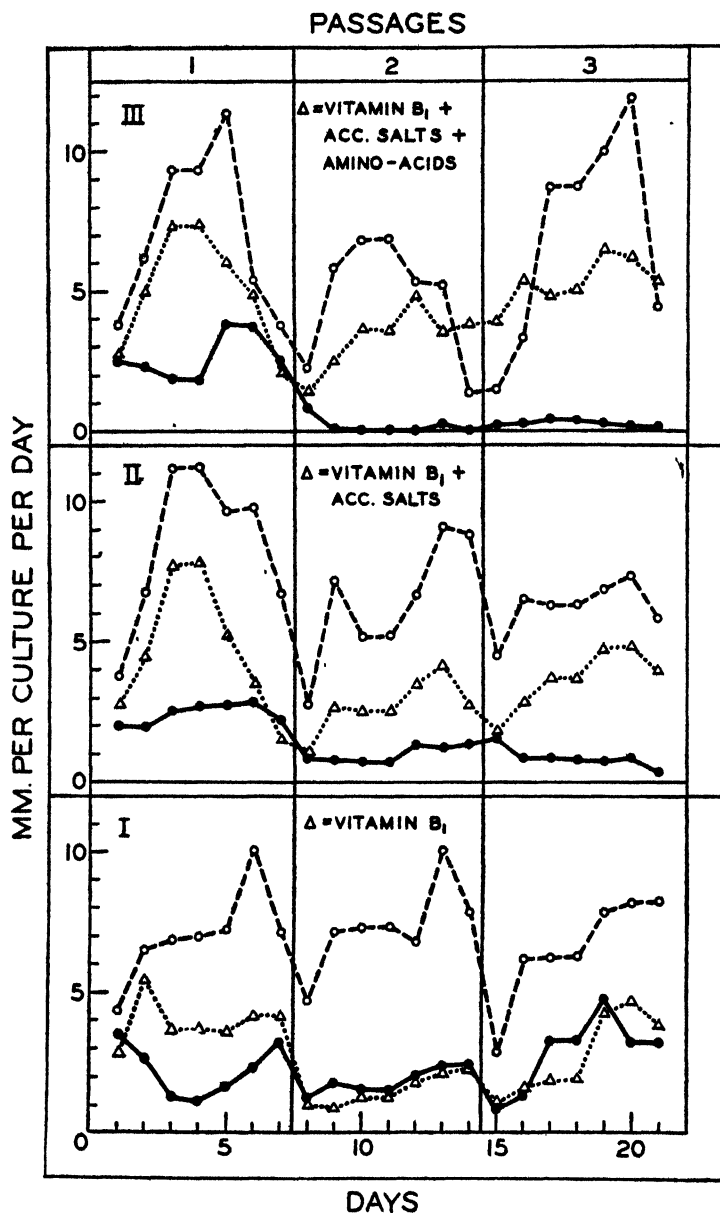


FIG. 2. Curves showing the growth rates of isolated tomato roots over three weeks' time in nutrients containing standard salts and sugar (solid circles) and with yeast extract added (open circles). The triangles represent growth in the same nutrient as that represented by the solid circles with the addition in I of vitamin B<sub>1</sub> at a concentration of 1.0 mg. per liter, of vitamin B<sub>1</sub> and accessory salts in II, and of vitamin B<sub>1</sub>, accessory salts, and amino acids in III.

Vitamin B<sub>1</sub> was added at concentrations of 1.0, 0.1, 0.01, and 0.001 mg. per liter when tested alone, and at these and the additional concentration of 0.0001 mg. per liter when tested in combination with the amino acids.

When vitamin B<sub>1</sub> alone was added to the basic nutrient, a marked increase in growth rate occurred over the controls without accessory material. The results were, however, far from uniform or optimal (figs. 1-II, 2-II, and 4). In the first passage, growth at vitamin B<sub>1</sub> concentration of 1 mg.

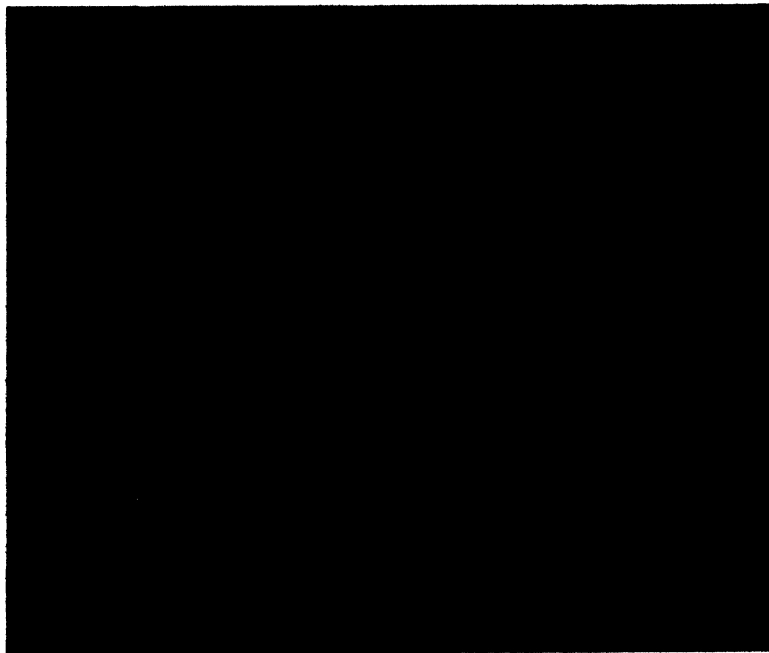


FIG. 3. Roots grown in a basic nutrient plus yeast (upper left), without any accessory material (upper right), and with addition of vitamin B<sub>1</sub> (lower row) at concentrations, reading from left to right, of 1.0, 0.1, 0.01, and 0.001 mg. per liter. Roots in the lower row are little if at all superior to the negative control.  $\times 0.7$ .

per liter was equal to 69 per cent. of the control in yeast extract. The growth rates decreased regularly (59 per cent., 56 per cent., 38 per cent.) with decreasing concentration of vitamin B<sub>1</sub>. In the second and third passages, a vitamin B<sub>1</sub> concentration of 0.1 mg. per liter gave the best results (52 per cent. and 72 per cent.). The roots were slender; most of the original growing points died so that growth occurred only in branches, and the branching was sparse. The condition of the cultures was obviously poor (fig. 4). A nutrient containing, in addition to the basic ingredients, only vitamin B<sub>1</sub>, while capable of sustaining growth for a considerable period—

at least three passages—was clearly not satisfactory as a replacement for yeast extract.

This result agrees with that reported by ROBBINS and BARTLEY (2). The growth obtained was, however, much better than that observed two years ago, as mentioned above. In examining the records of the two sets of experiments for possible explanations of this discrepancy, two differences between the sets were noted. In the first place, the early tests were carried out with "crystalline vitamin B<sub>1</sub> Merck" of natural origin, while the later ones were conducted with "Betabion Merck," a synthetic product. In the second place, the later tests were made in a nutrient which included "accessory salts" (5, 6), while these were absent from the nutrient used (4) in the earlier tests. It seemed improbable that growth-promoting materials present in the synthetic vitamin B<sub>1</sub> might be absent from the natural product. The presence or absence of the accessory salts, therefore, seemed the more likely cause of the discrepancy.

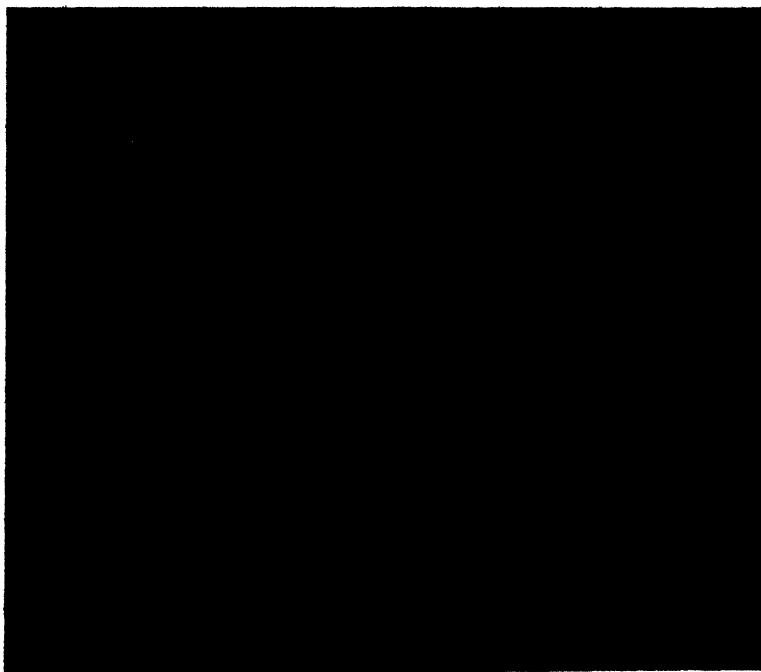


FIG. 4. Roots grown in nutrients similar to those used in cultures shown in figure 3, but with accessory salts added. Although the roots in the lower row are superior to the negative control (upper right), they are markedly inferior to those grown in the presence of yeast extract (upper left). It is clear that the tips in each experimental case had failed to grow and only a few branches were formed.  $\times 0.7$ .

To test this possibility, cultures were carried out in which vitamin B<sub>1</sub> was added at concentrations of 1.0, 0.1, 0.01, and 0.001 mg. per liter to a nutrient containing only the standard salts and sugar. In the first passage, while residual supplies of possible accessory salts carried over in the explant were still potentially available, the best growth, at a vitamin B<sub>1</sub> concentration of 1 mg. per liter, was equal only to 55 per cent. of the control (figs. 1-I, 2-I, and 3). In the second passage, growth at this concentration was only 21 per cent. of the control. In the third passage, growth at a concentration of 0.1 mg. per liter was equal to 55 per cent. of the control, but since the negative control, lacking all accessory material, likewise grew unusually well—42 per cent. of the control with yeast—this represents very little improvement over the completely deficient nutrient (figs. 2-I, and 3). From

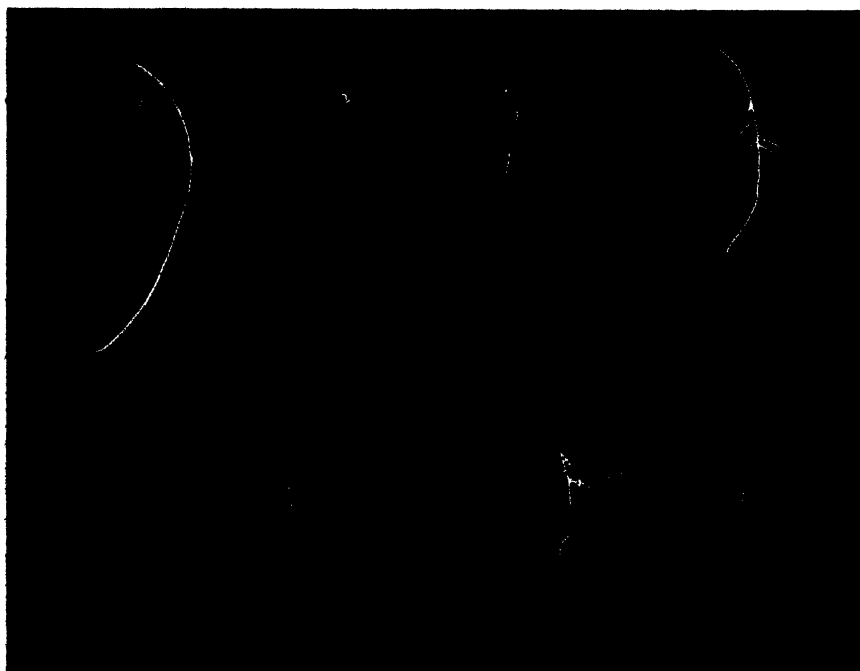


FIG. 5. Roots grown in the following nutrients: upper row, reading from left to right, control with standard salts, accessory salts, sugar, and yeast; the same but yeast omitted; the same but with an amino acid mixture substituted for the yeast; the same but with an absolute alcohol extract of yeast substituted for the usual yeast material; lower row, the same but with an amino acid mixture and vitamin B<sub>1</sub> at concentrations of  $10^{-8}$  to  $10^{-10}$  (from left to right) substituted for the yeast extract. The growing points of the experimental cultures have not died and the roots have branched quite profusely. While markedly superior to the roots grown without accessory organic material or with amino acids alone, or without accessory salts (fig. 3), they are still inferior to the controls provided with yeast.  $\times 0.7$ .

these results it is clear that the accessory salts were the *limiting* factor, the vitamin B<sub>1</sub> being unable to induce growth in their absence. The omission of accessory salts, therefore, was, as postulated above, responsible for failure to detect the effect of vitamin B<sub>1</sub> in earlier experiments. These results are in agreement with those of ROBBINS, WHITE, McCLARY, and BARTLEY (3).

When vitamin B<sub>1</sub> was similarly added to a nutrient containing the 9-amino-acid mixture developed in earlier work (6) in addition to the basic nutrient, the results were somewhat better than in a similar nutrient lacking the amino acids, but still far from optimal (figs. 1-III, 2-III, and 5). In all concentrations except 1 mg. per liter there was a regular diminution in growth rate from passage to passage, while at this concentration the growth rates in the three passages studied (71 per cent., 67 per cent., 75 per cent. of the control) were remarkably similar. The roots, while still slender and of reduced vitality (fig. 5), were much more profusely branched than when amino acids were omitted. Their color was better and the original growing points did not die. This nutrient, containing standard salts, accessory salts, sugar, vitamin B<sub>1</sub>, and amino acids, appeared to be capable of sustaining continuous growth of tomato roots at a level somewhat inferior to that obtained with yeast extract.

### Discussion

The work presented here has demonstrated that vitamin B<sub>1</sub> is of paramount importance in the nutrition of isolated tomato roots. This material is known to be present in yeast and is soluble in 100 per cent. alcohol. It was, no doubt, at least partly responsible for the activity of the yeast fraction extracted by this solvent. While concentrations of vitamin B<sub>1</sub> as low as 0.0001 mg. per liter gave a marked degree of stimulation, concentrations of 1.0 or 0.1 mg. per liter appeared to be somewhat superior. Since the yeast fraction soluble in 100 per cent. alcohol gave optimal results at a concentration of about 5 mg. per liter (5)—only slightly greater than this amount—it would appear that this fraction might consist largely of vitamin B<sub>1</sub>. Yet vitamin B<sub>1</sub> was *not* capable of completely replacing the fraction. Addition of amino acids (6) improved the result but still did not bring it up to the control. The roots cultivated in such a nutrient, while growing at a fairly uniform rate, were slender, crooked, and of reduced vitality. Some other material, soluble in 100 per cent. alcohol and effective at concentrations of less than 1 mg. per liter, while not *essential* for growth, must be responsible for the residual activity of this fraction.

The nutrient developed in this work, while inferior to yeast extract, does appear to be *complete*, in the sense of supporting *continuous* growth of tomato roots. And its constitution, except for possible impurities in the



"C.P. grade" chemicals used, is known and can be duplicated. One of the primary tasks in the study of growth requirements of isolated roots—the establishment of a completely known control nutrient containing all necessary growth factors—has then been provisionally completed. This nutrient, containing standard salts, accessory salts, sugar, vitamin B<sub>1</sub> at a concentration of 1.0 to 0.1 mg. per liter, and a 9-amino-acid mixture at a total concentration of 10 mg. per liter, may be used as a standard in future work.

While this nutrient appears to be "complete," its obvious deficiency when compared to a yeast extract medium raises certain important questions which must be left for future work to answer. Is the deficiency qualitative or quantitative? If it is only quantitative, a careful examination of the present ingredients of the mixture at higher and lower concentrations than at present used should serve to determine the optimal quantities. It is probable that in this process one or more ingredient out of the 18 salts and 9 amino acids at present included may prove to be unnecessary. If, however, as is equally likely, the deficiency is qualitative as well as quantitative, it will be more difficult to correct. The synthetic nutrient may be supplying materials which are capable, partly but not completely, of replacing some yeast material. The substitution of selenium for sulphur or of pyrrol for iron would be similar cases. If such is the case, the optimal concentrations of these "Ersatz-" materials would still give results inferior to those obtained with yeast extract. No amount of quantitative adjustment of the nutrient will correct the deficiency or even clearly indicate which ingredient is deficient, and more complex methods will have to be devised for solving the problem. To determine which is actually the case, a quantitative study of all the present ingredients of the nutrient is, therefore, indicated as the next step in approaching these questions.

### Summary

Experiments have shown that vitamin B<sub>1</sub>, a probable constituent of the yeast fraction soluble in absolute alcohol, is an important and perhaps indispensable factor in the nutrition of excised tomato roots. The growth-promoting effect of vitamin B<sub>1</sub> is detectable only in the presence of the "accessory salts" which are also indispensable. While growth at a low level can be maintained apparently indefinitely in a nutrient containing only vitamin B<sub>1</sub>, standard salts, accessory salts, and sugar, it is notably improved by the addition of a mixture of 9 amino acids.

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# DORMANCY AND GERMINATION OF *FRAXINUS* SEEDS

GEORGE P. STEINBAUER

(WITH SIX FIGURES)

## Introduction

The genus *Fraxinus* contains several species of woody plants of economic importance. The majority of these are propagated from seed in nature and under cultivation. Very little specific information is available relative to the factors affecting seed germination in the different species.

Special treatments of the seeds are often required to produce uniform stands of seedlings in the year following harvest of the seed. The most common recommendations for germinating *Fraxinus* seeds suggest stratification of the seeds at 5°–10° C. previous to planting (8, 11). In many instances, untreated seeds may remain dormant in the soil for one or more years after planting. PUCHNER (10) reported one case in which seeds of European ash, *Fraxinus excelsior* L., remained in a dormant condition in the soil for six years before they germinated.

Information on the causes of delayed germination of *Fraxinus* seeds is fragmentary and meager. The most thoroughly studied case is that of the seeds of European ash (4, 9) in which the embryo is small, though morphologically complete at the maturity of the seed, and requires a period of time for enlargement before the enveloping tissues and coats can be broken. Mechanical resistance of the enveloping tissues has been suggested as a possible reason for further delay in germination. Studies on green ash seed have suggested a dormant condition of the embryo (7).

The present study has been confined to the following aspects of the problem: first, the relative importance of embryo and enveloping tissues in causing delayed germination; second, the factors affecting the growth of excised and encased embryos; third, the effectiveness of current stratification procedures in aiding germination of the seeds of different species; and fourth, the factors affecting the vitality of stored seeds.

Seeds of the following four species were used as material for this study: white ash, *Fraxinus americana* L.; black ash, *Fraxinus nigra* Marsh.; red ash, *Fraxinus pennsylvanica* Marsh.; and green ash, *Fraxinus pennsylvanica* var. *lanceolata* (Borkh.) Sarg. The above species are of commercial importance, grow in somewhat different habitats, and produce seed in the vicinity of Orono, Maine. The data presented here should be considered as applying primarily to the species studied, although there is some evidence that these results may apply also to other species.

## Observations

### STRUCTURE OF THE SEED AND FRUIT OF *FRAXINUS*

Since the course of the germination process depends largely upon the structure of the seed, a brief description of it is essential. In *Fraxinus* the unit commonly spoken of as the "seed" is in reality a winged fruit or samara. The true seed is exposed only after the ovary wall or pericarp has been removed. The fruits, seeds, and embryos of red and black ash are shown in figure 1.



FIG. 1. The fruit, seed, and embryo of *Fraxinus*. A. Red ash. B. Black ash. From left to right: fruit, seed, embryo. Approximately normal size.

The extent and location of the more important parts of the samara are shown diagrammatically in figure 2, using the fruit of black ash as an example. Usually one seed is produced within each fruit although two and even three seeds are not uncommon.

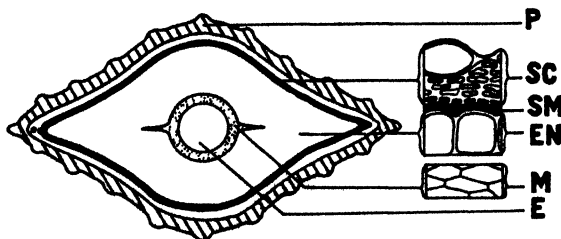


FIG. 2. Cross section of the fruit of black ash (diagrammatic). At the right a few cells are shown from the layers of the seed external to the embryo. Cell contents have been omitted.

P—pericarp  
SC—seed coats  
SM—suberized membrane

EN—endosperm  
M—mucilaginous layer  
E—embryo

The seed coats, SC, are made up of five to eight layers of cells. The innermost cells of the seed coats are separated from the endosperm cells, EN, by a membrane, SM. The exact chemical composition of the latter has not been determined although staining properties, solubility, and other microchemi-

cal tests indicate the presence of some cutin and suberin. This membrane is one of the most resistant layers external to the embryo, and very likely plays an important part in preventing rapid expansion of the embryo.

The reserve food materials of the endosperm consist largely of fats and reserve proteins. Starch and soluble carbohydrates appear in the endosperm and embryo in the later stages of the germination process.

The embryo, E, is surrounded by a layer of cells, M, containing mucilaginous material, called the "schleimschicht" by LAKON (9) relative to the seeds of European ash. These cells have thinner walls and less reserve food material than those of the endosperm. Owing to the semi-gelatinous consistency of this layer, the embryos of *Fraxinus* seeds may be easily excised without injury.

The degree of embryo development at maturity of the seed varies considerably among the different species. The embryos are fully differentiated into hypocotyl, cotyledons, and epicotyl, but the relative size of embryo and seed varies widely. In figure 1A there is illustrated the type of seed in which the embryo extends the full length of the seed. The seeds of red, white, and green ash are of this type. Such seeds are quite easily germinated if they are first stratified for a period of one or two months at a temperature near 5° C. In figure 1B there is illustrated the type of seed in which the embryo extends from one-half to two-thirds the length of the seed. Seeds of European ash are said to be similar to those of black ash in this respect (9). Such seeds do not germinate readily. They do not respond favorably to ordinary stratification treatments and in nature often remain in a dormant condition in the soil for one or more years before they germinate (5, 9).

#### DORMANCY AND GROWTH OF EXCISED EMBRYOS OF BLACK ASH

Since the embryos of black ash are much smaller in proportion to the rest of the seed than those of the other species studied, it seemed desirable to make observations on them first.

In order to determine whether or not embryos of recently harvested seeds are dormant, a number of embryos were excised from soaked seeds. They were placed on moist blotting paper in Petri dishes and kept at room temperature. At the end of two weeks there was no evidence of growth in such excised embryos.

Various treatments were then used in an attempt to overcome this initial dormancy. These treatments included different rates of aeration, use of light, various substrata, and a series of different temperatures. The use of higher than room temperatures was effective in inducing growth. Thus at the end of four days some of the embryos in the 30°-C. chamber were beginning to show geotropic curvature of the hypocotyl. This was followed by elongation of the cotyledons and the formation of root hairs. When these

seedlings were planted in soil in an illuminated moist chamber, they soon formed chlorophyll. Owing to the limited supply of reserve food contained in the embryo, they remained in a dwarfed condition for some time but eventually developed into normal plants.

The necessity for higher-than-room temperatures in breaking this initial dormancy is evident from table I, which shows the results obtained when lots of sixty embryos each were kept at temperatures of 5°, 20°, 25°, and 30° C. It is evident that dormancy is not equally deep seated in all embryos held at a given temperature. The results explain, in part, why stratification at low temperatures, which is so successful with some species of *Fraxinus*, is ineffective for seeds of black ash.

TABLE I

EFFECTIVENESS OF VARIOUS TEMPERATURES IN BREAKING THE DORMANCY OF EXCISED EMBRYOS  
OF BLACK ASH, *FRAXINUS NIGRA*

DURATION OF TEST	NUMBER OF EMBRYOS SHOWING GEOTROPIC CURVATURE (60 EMBRYOS BASIS OF RESULTS AT EACH TEMPERATURE)			
	5° C.	20° C.	25° C.	30° C.
<i>days</i>				
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	6
5	0	0	0	6
6	0	0	0	8
7	0	0	0	10
8	0	0	0	16
9	0	0	0	22
10	0	0	0	24
15	0	0	8	44
20	0	0	12	49
25	0	0	18	60
30	0	4	18	60

#### GROWTH OF ENCASED EMBRYOS OF BLACK ASH SEEDS

Although it is possible to produce plants from excised embryos of recently harvested seed, in nature the embryos undergo considerable enlargement before visible evidence of germination appears. LAKON (9), who reported a similar type of embryo development in the seeds of European ash, suggested the use of the term "Vorkeimung" to distinguish the growth changes of such embryos from the chemical and physical changes involved in "after-ripening" of seeds. The relative sizes of embryos at the beginning and end of this growth period are shown in figure 3. It is obvious that the small em-

bryos of recently harvested seeds are not comparable to the rudimentary embryos described by CROCKER (1) in *Corydalis*, *Ranunculus*, and other seeds. Since the time required for embryo enlargement may be weeks, or even months, it is important to know the optimum conditions for growth of the embryo.

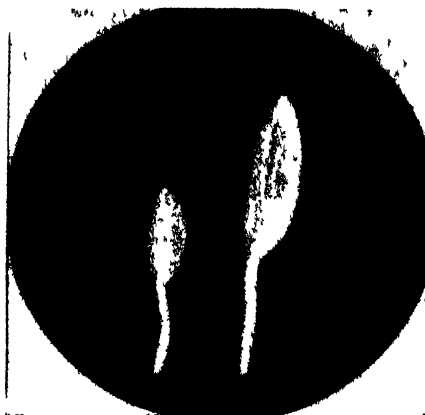


FIG. 3. Enlargement of the black ash embryo previous to germination. Left, embryo at time of shedding of the seed. Right, embryo just previous to protrusion of the radicle in germination. About  $2 \times$  normal size.

The length of time required for the embryos to attain their maximum size depends to a large degree upon the temperature of the germination

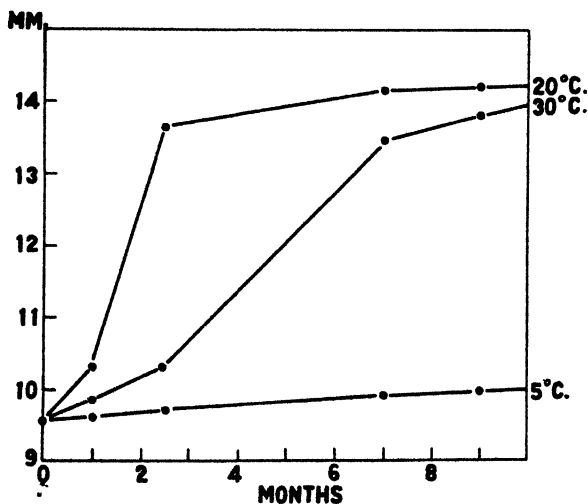


FIG. 4. Graph showing the influence of temperature on growth of encased embryos of black ash. Lengths of embryos expressed in millimeters are averages of one hundred measurements at each temperature.



chamber. Seeds were placed between layers of moist linen toweling and stored at temperatures of 5°, 20°, 25°, and 30° C. Embryos were excised from these different lots of seed at the end of four, ten, twenty-eight, and thirty-six weeks and measured for increases in length. The results obtained are shown graphically in figure 4. Measurements made on embryos held at 25° C. were intermediate between those of the 20° and the 30° C. groups and have been omitted from the graph.

The growth curve during this period of embryo development exhibits three rather distinct phases. The slow growth at first may be accounted for by the initial embryo dormancy mentioned above. This is followed by a period of rapid elongation of both cotyledons and hypocotyl. When the embryos have attained a length of 14–14.5 mm., the rate of growth rapidly decreases. Maximum size is attained at between two and three months at 20° C., and considerably later at 25° and 30° C. Further storage of seeds containing enlarged embryos at temperatures of 20° C. or higher does not ordinarily result in germination. One lot of seed stored at 20° C. had fully enlarged embryos at the end of two months and yet failed to produce a single seedling during the following two years of storage at 20° C.

That germination of seeds with fully enlarged embryos is not due to embryo dormancy is shown by the behavior of excised embryos. The latter will often show marked geotropic curvature within twelve hours after excision, which is soon followed by elongation of cotyledons and the formation of root hairs. By making a longitudinal incision in the seed, being careful not to injure the embryo, one can often induce germination within a few days. This suggests that the delay in germination is due to restrictions placed upon the embryo by the enveloping layers: endosperm, suberized layer, and seed coats. The pericarp probably is not an important factor since it disintegrates readily at temperatures of 20° C. or higher.

The retarding influence of the enveloping tissues on further growth of the embryo seems to be primarily in the nature of mechanical resistance. In this connection it is interesting to note that further growth of the cotyledons is not retarded as much at the higher temperatures as is the growth of the hypocotyl. In this respect the embryos are somewhat similar to the embryos of *Crataegus* (3) and *Ambrosia* (2). Abnormal germination is not uncommon in the seeds of black ash and in other species of ash. The appearances of *Fraxinus* seeds exhibiting normal and abnormal germination are shown in figure 5.

It is interesting to note that 5° C. is not conducive to embryo enlargement although it is a very effective temperature in forcing germination after the embryos have become fully enlarged. Likewise it is interesting to note that whereas 30° C. is a highly effective temperature for the growth of excised embryos it is less effective than 20° C. when the embryos are still within the seed.



FIG. 5. Normal and abnormal germination of *Fraxinus* seeds. Left, abnormal germination of red ash seed. Right, normal germination of white ash seeds. Note that in abnormal germination the cotyledons protrude before the hypocotyl whereas in normal germination the reverse takes place.

#### GERMINATION OF SEEDS CONTAINING FULLY ENLARGED EMBRYOS

Since temperatures favorable to enlargement of the embryo fail to promote normal germination, seeds with fully enlarged embryos were stored in a moist condition at temperatures of 5°, 20°, 30° C. and observed as to further changes in the embryos. Microchemical studies on seeds stored at these different temperatures indicated that 5° C. was the most favorable temperature for the various processes of digestion. Seeds stored at this temperature were characterized by a more rapid accumulation of starch, soluble carbohydrates, and proteins in the embryo than was the case with embryos of seeds stored at higher temperatures.

Excised embryos taken from these different lots of seeds also showed considerable variation in their capacity to resume growth. These differences are shown in figure 6. In this experiment embryos excised from seeds held at 5° C. exhibited geotropic curvature within ten hours after excision. Embryos from seeds stored at 20° C. required 24 hours to reach the same stage of development, and those from the 30° C. lot of seed were still in their original condition at the end of four days.

Although protrusion of the radicle occurs only rarely at 5° C., the digestion of reserves of the endosperm and the accumulation of these materials in the embryo make possible rapid growth and germination when the seeds are transferred to higher temperatures.

The following scheme summarizes the principal events leading up to the protrusion of the radicle through the seed and fruit coats. Optimum temperatures for the different stages of the germination process are indicated.

Seeds with small, morphologically complete embryos.  
Dormancy of excised embryos broken by temperatures of 25°–30° C.



I

### EMBRYO ENLARGEMENT

Most effective temperature: 20° C. for 2–3 months.  
Moderate rate of growth of excised embryos at this temperature.  
Digestion slow.  
Very little accumulation of food in embryo.

Enlargement  
of embryo

Restrictions placed on further growth by enveloping tissues and membranes.  
Mechanical resistance and possibly other factors involved.  
Excised embryos not dormant. Normal germination rare at this temperature. Abnormal germination occasionally occurs.



II

### CHANGES IN SEED PRODUCED BY LOW TEMPERATURE STRATIFICATION

Most effective temperatures: 5°–10° C. for 2–3 months.  
Growth of excised embryos very slow at this temperature.  
Digestion continues. Depletion of fat and other reserve foods of endosperm. Accumulation of starch, soluble carbohydrates, and proteins in embryo.  
Excised embryos not dormant. Germination may occur in seeds with more vigorous embryos.



III

### GERMINATION

Most effective temperatures: Daily alternation of 20°–30° C.  
Growth of excised embryos rapid at this temperature.  
Supply of food accumulated in embryos in stage II combined with high temperatures makes possible sufficient pressure (osmotic, imbibitional, etc.) to overcome mechanical resistance of layers external to embryos.

Seedlings may be produced from excised embryos at either stage, most easily at late I or in II.

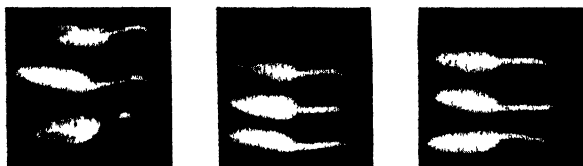


FIG. 6. Effect of storage temperatures on behavior of excised embryos of black ash seeds. Left, embryos from 5° C. chamber. Center, embryos from 20° C. chamber. Right, embryos from 30° C. chamber.

#### GERMINATION OF SEEDS OF RED, GREEN, AND WHITE ASH

Studies on seeds of red, green, and white ash indicated that embryos excised from freshly harvested seed are dormant. In the case of red and green ash the period of dormancy can usually be reduced to a few days if the temperature of the germination chamber is maintained near 30° C. Embryos excised from white ash seeds remained dormant over a longer period of time and required a lower temperature for resumption of growth than did the embryos of the other species that were studied.

The most favorable conditions for germination of the seeds of red, green, and white ash are quite different from those required by seeds of black ash. Placing the seeds in germinators at temperatures at or near room temperature was ineffective in producing uniform germination. Abnormal germination as previously described occasionally occurs. The seeds of these species require approximately the same temperatures for germination as do the seeds of black ash at the time when the embryos have become fully enlarged. Thus the most uniform results are obtained when the seeds are stored in a moist condition for two or three months at 5°–10° C. and then transferred to a chamber with a higher temperature such as 20° C. or 30° C.

#### SUGGESTED PLANTING PRACTICES

The most uniform stands of seedlings can be obtained in the nursery or plantation by taking into account the temperature requirements for germination of the different species. In the case of red, white, and green ash very good results were obtained by fall planting immediately after the seeds had been harvested. This procedure allows the seeds to be subjected to moisture and low temperature during fall and early spring.

Occasionally the same results may be obtained if seeds that have been stored over winter in a dry condition are planted out of doors very early in the spring, so as to take advantage of the low temperatures then prevalent. If planting of dry seeds is delayed too long, the warm weather may prevent germination during the same season. If fall or early spring planting is not practiced, stratification of the seeds for two or three months at 5°–10° C. previous to planting is the best procedure.

Successful propagation of black ash seedlings from seed must take into account the conditions required for enlargement of the embryo. Fall plantings of black ash seed in the Maine State Forestry Nursery at Orono, Maine, have so far been unsuccessful in producing seedlings the following season. Germination occurred in the second spring after the planting of the seeds.

The planting of dry seeds in the spring is almost certain to result in failure to obtain a crop of seedlings until the following season. Stratification procedures commonly recommended for red, green, and white ash seeds should be preceded by a period of two or more months' storage of the seeds at a temperature near 20° C. This allows the embryos to become fully enlarged, after which the seeds may be treated in the same manner as those of red and green ash.

#### EFFECT OF STORAGE CONDITIONS ON VITALITY OF ASH SEEDS

Since it is often desirable to defer planting of the seeds until the spring following the harvesting of the seeds, or occasionally to store them for more than one year, it is important to know the optimum storage conditions. The seeds of the majority of plants retain their vitality best when kept dry and at a low temperature. A few seeds, such as those of the maple (6), require a considerable amount of moisture along with a favorable temperature in order to remain viable.

In order to determine the most favorable storage conditions, aliquot samples of each of the different kinds of ash seed were stored in sealed containers. In one series the seeds were placed in sealed containers over dehydrating agents so selected as to yield various known relative humidities. In the second series the seeds were kept at their original moisture contents but at temperatures ranging from 5°–30° C. The results of these different treatments in terms of percentage of germination in the nursery beds are given in table II. Results for green and white ash seeds were similar to those for red and black ash seeds.

It is evident from the table that the seeds of these species are much more sensitive to changes in moisture content than to changes in temperature. Desiccation of the seeds to within 1.5 per cent. of their oven-dry weight did not appear injurious. The critical relative humidity of the storage chamber appears to be between 50 and 75 per cent. If the moisture content of the seeds can be reduced to about 7.5 per cent. of their dry weight and the seeds stored in closed containers, or if the storage room can be kept at a relative humidity of 50 per cent. or less, the seeds may be expected to retain their vitality for at least one or two seasons after harvest. Studies on excised embryos indicated that the reduction in the percentage of germination resulting from storage at high relative humidities was due to a deterioration of the embryo rather than to changes in the enveloping tissues and membranes.

TABLE II  
VITALITY OF *FRAXINUS* SEEDS IN RELATION TO STORAGE CONDITIONS

STORAGE CONDITIONS*	BLACK ASH <i>FRAXINUS NIGRA</i>		RED ASH <i>FRAXINUS</i> <i>PENNSYLVANICA</i>	
	MOISTURE CONTENT	GERMI- NATION†	MOISTURE CONTENT	GERMI- NATION†
	%	%	%	%
Over concentrated sulphuric acid	1.72	38	1.68	53
25 per cent. relative humidity } 25° C.	5.55	46	5.36	48
50 " " " " }	7.33	39	6.49	50
75 " " " " }	11.39	0	9.85	1
5° C. ....	7.50	40	7.30	54
20° C. ....	"	50	"	49
25° C. ....	"	43	"	56
30° C. ....	"	38	"	51

\* Storage of seeds for one year in sealed containers previous to planting.

† Figures based on quadruplicate samples of 100 seeds each.

### Summary

1. A study of the optimum conditions for germination of the seeds of red, green, white, and black ash indicates that the seeds of black ash do not germinate at temperatures favorable to germination of the other species.

2. The differences in temperature requirements of the various species of ash depend in part upon the size of embryo at maturity of the seed.

3. Embryos of black ash seeds undergo considerable enlargement before germination occurs. Temperatures near 20° C. are most effective in promoting growth during this period.

4. Germination of the seeds of black ash does not occur at temperatures favorable to enlargement of the embryo. Further growth of the embryo is retarded by mechanical resistance of the enveloping tissues and coats.

5. Stratification of black ash seeds at 5° C. enables the fully enlarged embryos to absorb and accumulate reserve materials from the endosperm. After a period of two or three months stratification, the embryos are able to overcome the resistance of the enveloping membranes when placed at higher temperatures.

6. Embryos of red, green, and white ash are fully enlarged at maturity of the seed. Stratification of the seeds at 5° C. brings about changes favorable to germination.

7. Dwarf seedlings may be obtained from embryos excised from freshly harvested seeds of all of the species studied.

8. Storage of seeds in sealed containers at different temperatures and relative humidities showed that vitality of the seeds is retained the longest

when the seeds are kept at a moisture content of less than 7.5 per cent. of the dry weight. Seeds with a moisture content of less than 7.5 per cent. retained their vitality equally well over a wide range of temperatures.

9. Suggestions are given for effective planting procedures for the different species studied.

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# INFLUENCE OF HOST NUTRITION ON SYSTEMIC DEVELOPMENT OF TOBACCO MOSAIC

ERNEST L. SPENCER

In two previous papers it was shown (3, 4) that the mineral nutrition of tobacco plants had a marked effect on their susceptibility to primary infection with a yellow strain of tobacco-mosaic virus. In the present paper the influence of host nutrition on systemic infection of this same virus in infected tobacco plants will be discussed.

Several workers have studied the movement of virus in plants, but little attention has been given to the influence which host nutrition may have on systemic spread of virus. BÖNING (1), in 1928, published the results of an experiment dealing with the effect of nitrogen nutrition on mosaic-virus spread in tobacco. The tip of the largest leaf on each plant was inoculated. With actively growing plants an average of 3 days was required for the virus to pass 13 cm. from the tip of the leaf to the stem. With slow-growing, nitrogen-deficient plants an average of at least 6 days was necessary for the virus to pass from a leaf 12 cm. long. BÖNING concluded that the rapidity of virus-spread from an inoculated leaf was greatly decreased by nitrogen deficiency. VOLK (5) studied the effect of potassium, phosphorus, and nitrogen on the spread of streak in tomato plants grown in nutrient sand cultures, and inoculated just prior to fruiting. He concluded that the spread of streak disease was most rapid and the effect of the disease most severe in plants fertilized with an ample supply of potassium and phosphorus or in plants deficient in nitrogen.

## Experimentation

### MATERIALS AND METHODS

Turkish tobacco (*Nicotiana tabacum* L.) was used as the experimental host. Seeds were germinated in quartz sand and kept moist with a dilute nutrient solution. When the seedlings had reached a height of about 2 cm. and had developed 3 or 4 small leaves, plants were selected for uniformity of size and potted in 4-inch porous clay pots filled with washed, quartz sand. The pots were then placed in saucers on a greenhouse bench and were spaced so as to avoid undue crowding of foliage. During the course of the experiments the greenhouse temperature was held between 70° and 80° F. during the day and between 70° and 75° F. at night. The greenhouse was fumigated with nicotine at weekly intervals to prevent accidental inoculation by insect vectors.

Nutrient treatments were usually started 3 days after the seedlings had been potted. Each seedling received 100 cc. of nutrient solution 3 times each



TABLE I  
COMPOSITION OF NUTRIENT SOLUTIONS

NUTRIENT SOLUTIONS	VOLUME OF 0.5 MOLAR STOCK SOLUTIONS REQUIRED TO PRODUCE 1 LITER OF NUTRIENT SOLUTION							
	$\text{KH}_2\text{PO}_4$	$\text{Ca}(\text{NO}_3)_2$	$\text{MgSO}_4$	$(\text{NH}_4)_2\text{SO}_4$	$\text{NH}_4\text{H}_2\text{PO}_4$	$\text{KNO}_3$	$\text{K}_2\text{SO}_4$	$\text{CaCl}_2$
	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
Nitrogen								
Minus ..	12.9	...	4.0	.....	.....	.....	.....	7.8
Medium	12.9	7.8	4.0	3.6	.....	.....	.....	...
High .....	.....	96.5	4.0	33.5	12.9	12.9	.....	.....
Phosphorus								
Minus ..	.....	11.0	4.0	3.3	.....	.....	48.4	.....
Medium	12.9	11.0	4.0	3.3	.....	.....	42.0	.....
High .....	96.8	11.0	4.0	3.3	.....	.....	.....	.....
Potassium								
Minus ..	.....	7.8	4.0	.....	12.9	.....	.....	.....
Medium	12.9	11.0	4.0	3.6	.....	.....	.....	.....
High .....	12.9	7.8	4.0	6.5	.....	.....	121.4	.....

week. The composition of these solutions is given in table I. In each case the "medium" solution served as a check on the "minus" and "high" solutions and was of such a composition as to produce healthy, vigorous growth. The "high" solution contained the element in question at such a level that growth was retarded but not entirely inhibited. The "minus" and "high" solutions were used in order that a study could be made of the action exerted not only by a deficiency but also by an excess of the element in question. In addition to the salts listed in table I, boron as  $\text{H}_3\text{BO}_3$ , and manganese as  $\text{MnSO}_4 \cdot 2\text{H}_2\text{O}$  were added in concentrations equivalent to 0.5 p.p.m. of each element to all culture solutions.

In each experiment the nutrient treatment was as follows: The seedlings were divided into 7 groups. One group received the "medium" solution throughout the time of nutrient application. During the first week of nutrient treatment, 1 of the 6 remaining groups received the "high" solution and 5 received the "minus" solution. At the end of each week one group of seedlings was transferred from the "minus" to the "high" solution. Thus, at the time of inoculation, 4 weeks after the initial nutrient treatment, one group had received the "high" solution for 1 week, a second group for 2 weeks, a third group for 3 weeks, and a fourth group for 4 weeks. This arrangement left 2 groups on the "minus" solution, but at the time of inoculation 1 of these 2 groups was transferred to the "high" solution. All groups received their respective nutrient solutions for 1 week following inoculation and then were watered whenever necessary with tap water until the experiment was terminated. At time of inoculation, the green weight and height of representative plants in each group were determined in order to obtain criteria of comparative growth.

The virus used was a yellow strain of tobacco-mosaic virus, formerly designated by JOHNSON (2) as tobacco virus 6. Representative plants in each treatment were inoculated by rubbing the tip portion of a leaf situated about half-way up the stem with undiluted juice from a diseased tobacco plant. All plants inoculated showed many yellow primary lesions on the inoculated leaves, but no attempt was made to count them. Following inoculation, the time required for the virus to reach and produce symptoms on leaves at the top of the plant was noted. When systemic infection appeared shortly after inoculation, the first symptom was a clearing of veins. When systemic infection appeared only after many days, the first symptoms were yellow lesions with indistinct, irregular margins. This stage was followed by clearing of veins.

#### INFLUENCE OF NITROGEN NUTRITION ON SYSTEMIC INFECTION

The nitrogen series was started in September and extended into November, 1936. In this series the nutrient solutions, the compositions of which are given in table I, all contained 20 mg. of phosphorus and 25 mg. of potassium per 100 cc. of solution. The medium-nitrogen solution contained 16 mg. of nitrogen, and the high-nitrogen solution 200 mg. per 100 cc. of solution. Each of the 7 groups contained 48 plants. After 4 weeks of nutrient treatment, 30 representative plants were selected in each group and inoculated as previously described. Table II gives the experimental data obtained in this test on the influence of nitrogen nutrition on growth and systemic infection of the virus. The green weights, based on an average

TABLE II  
EFFECT OF NITROGEN ON SYSTEMIC SPREAD OF A YELLOW STRAIN OF  
TOBACCO-MOSAIC VIRUS

NITROGEN ADDED EACH WEEK (MG./POT)					GROWTH RECORDS AT TIME OF INOCULATION*		PERCENTAGE OF PLANTS SHOWING SYSTEMIC INFECTION	NUMBER OF DAYS ELAPSED BEFORE AP- PEARANCE OF SYSTEMIC SYMPTOMS
1ST WEEK	2ND WEEK	3RD WEEK	4TH WEEK	5TH WEEK	GREEN WEIGHT	HEIGHT		
mg.	mg.	mg.	mg.	mg.	gm.	cm.	%	days
0	0	0	0	0	1.4†	1†	100	6.43‡ ± 0.26§
0	0	0	0	600	1.4	1	100	5.03 ± 0.03
0	0	0	600	600	4.9	2	100	5.00 ± 0.00
0	0	600	600	600	7.0	3	100	5.10 ± 0.06
0	600	600	600	600	14.6	8	100	5.77 ± 0.09
600	600	600	600	600	15.9	9	100	6.43 ± 0.21
48	48	48	48	48	32.7	28	100	21.27 ± 0.93

\* Plants inoculated at end of 4th week.

† Average of 10 plants in each group.

‡ Average of 30 plants in each group.

§ Standard error of the mean.

of 10 plants in each group and determined at time of inoculation, show that the plants which received the minus-nitrogen solution made very little growth. Plants which received 600 mg. of nitrogen each week for 3 or 4 weeks made much better growth, although they were only about one-half the size of those which received 48 mg. of nitrogen each week. All inoculated plants, with the exception of those which received 48 mg. of nitrogen each week, showed symptoms of systemic infection within 10 days. There were only slight differences among the groups which received no nitrogen or an excess of nitrogen in the time required for the development of systemic symptoms. When no nitrogen was added, an average of more than 6 days elapsed before the development of such symptoms. When the high-nitrogen solution was first added at time of inoculation, an average of 5 days elapsed before the appearance of symptoms of systemic infection. Even when 600 mg. of nitrogen were added each week for 4 weeks prior to inoculation, about 6 days elapsed before the appearance of symptoms. However, when 48 mg. of nitrogen were added each week, an average of more than 21 days elapsed before symptoms of systemic infection developed. From these data it is apparent that the absence of nitrogen or the presence of relatively large quantities of nitrogen in the nutrient solution increased the rapidity of development of symptoms of systemic infection.

#### INFLUENCE OF PHOSPHORUS NUTRITION ON SYSTEMIC INFECTION

The phosphorus series was carried out during March and April, 1936. The compositions of the nutrient solutions used are given in table I. Each of these solutions contained 20 mg. of nitrogen and 190 mg. of potassium per 100 cc. of solution. The medium-phosphorus solution contained 20 mg. of phosphorus, and the high-phosphorus solution 150 mg. per 100 cc. of solution. Each of the 7 groups contained 48 plants. Thirty-six plants in each group were inoculated as previously described. The experimental data obtained in this study of the effect of phosphorus nutrition on growth and systemic infection are shown in table III. From the green-weight data, based on an average of 12 plants in each group, it is apparent that growth increased with the increasing period of time during which the high-phosphorus solution was added, but in no case did plants which received 450 mg. of phosphorus each week make as good growth as those which received 60 mg. each week. All inoculated plants showed symptoms of primary infection at the site of inoculation, but several of them failed to show symptoms of systemic infection. The data in the last column in table III give the average number of days which elapsed before the development of symptoms on the 28 plants which first showed systemic infection in each group. The phosphorus-deficient plants showed systemic infection in about 7 days. Plants that had received 450 mg. of phosphorus during the week prior to

TABLE III

EFFECT OF PHOSPHORUS ON SYSTEMIC SPREAD OF A YELLOW STRAIN OF  
TOBACCO-MOSAIC VIRUS

PHOSPHORUS ADDED EACH WEEK (MG./POT)					GROWTH RECORDS AT TIME OF INOCULATION*		PERCENTAGE OF PLANTS SHOWING SYSTEMIC INFECTION	NUMBER OF DAYS ELAPSED BEFORE AP- PEARANCE OF SYSTEMIC SYMPTOMS
1ST WEEK	2ND WEEK	3RD WEEK	4TH WEEK	5TH WEEK	GREEN WEIGHT	HEIGHT		
mg.	mg.	mg.	mg.	mg.	gm.	cm.	%	days
0	0	0	0	0	11.6†	7†	100	7.18† ± 0.17§
0	0	0	0	450	11.6	7	100	6.46 ± 0.11
0	0	0	450	450	16.0	7	100	7.75 ± 0.17
0	0	450	450	450	20.1	10	97	12.29 ± 0.78
0	450	450	450	450	28.3	15	97	16.79 ± 0.62
450	450	450	450	450	33.2	19	78	17.96 ± 0.82
60	60	60	60	60	38.6	24	81	19.36 ± 0.88

\* Plants inoculated at end of 4th week.

† Average of 12 plants in each group.

‡ Average time for development of symptoms on the 28 plants which first showed systemic infection in each group.

§ Standard error of the mean.

inoculation showed systemic infection in about 8 days. Those that had received 450 mg. of phosphorus each week for 2 weeks prior to inoculation showed systemic infection in about 12 days. Those that had received this application of phosphorus for 3 weeks prior to inoculation showed systemic infection in about 17 days. Of the 36 plants that had received 450 mg. of phosphorus each week for 4 weeks prior to inoculation, only 28 showed systemic infection within 30 days after inoculation. The symptoms developed on these 28 plants within an average of about 18 days. With the addition of medium amounts of phosphorus (60 mg. each week), an average of 19.4 days elapsed before the appearance of systemic infection. It is evident, therefore, that the addition of medium or high amounts of phosphorus interfered in some way with systemic spread of the virus through the host or the subsequent development of systemic symptoms.

#### INFLUENCE OF POTASSIUM NUTRITION ON SYSTEMIC INFECTION

The potassium series was carried out during October, November, and December of 1935. The compositions of the nutrient solutions used in this series are given in table I. Each of these solutions contained 20 mg. of nitrogen and 20 mg. of phosphorus per 100 cc. The medium-potassium solution contained 25 mg. of potassium, and the high-potassium solution 500 mg. per 100 cc. of solution. Each of the 7 groups contained 36 plants. Twenty-five representative plants in each group were inoculated as previously described. In table IV are recorded the experimental data derived

**TABLE IV**  
**EFFECT OF POTASSIUM ON SYSTEMIC SPREAD OF A YELLOW STRAIN OF**  
**TABACCO-MOSAIC VIRUS**

POTASSIUM ADDED EACH WEEK (MG./POT)					GROWTH RECORDS AT TIME OF INOCULATION*		PERCENTAGE OF PLANTS SHOWING SYSTEMIC INFECTION	NUMBER OF DAYS ELAPSED BEFORE APPEARANCE OF SYSTEMIC SYMPTOMS
1ST WEEK	2ND WEEK	3RD WEEK	4TH WEEK	5TH WEEK	GREEN WEIGHT	HEIGHT		
<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>gm.</i>	<i>cm.</i>	<i>%</i>	<i>days</i>
0	0	0	0	0	19.4†	20†	100	9.36‡ ± 0.69§
0	0	0	0	1500	19.4	20	100	8.48 ± 0.56
0	0	0	1500	1500	20.3	20	100	14.80 ± 1.09
0	0	1500	1500	1500	19.4	18	100	17.88 ± 1.26
0	1500	1500	1500	1500	18.5	18	100	19.96 ± 1.20
1500	1500	1500	1500	1500	15.7	14	100	18.08 ± 0.89
75	75	75	75	75	29.1	30	100	13.68 ± 1.05

\* Plants inoculated at end of 4th week.

† Average of 8 plants in each group.

‡ Average of 25 plants in each group.

§ Standard error of the mean.

from the potassium series. The retardation in growth resulting from the addition of the high-potassium solution for 3 or 4 weeks was slightly more than that brought about by the addition of the potassium-deficient solution. The potassium-deficient plants showed symptoms of potassium deficiency in their lowermost leaves, whereas the high-potassium plants showed no signs of injury except the retardation in growth. All of the inoculated plants showed symptoms of systemic infection within 30 days. In plants grown with the potassium-deficient solution, symptoms of systemic infection appeared within an average of 10 days. The addition of high potassium at time of inoculation further shortened the average time required for the appearance of systemic infection by about 1 day. When high potassium was added 1 week prior to inoculation, symptoms appeared within about 15 days after inoculation. When the high-potassium solution was added for 2 or more weeks before inoculation, symptoms appeared within 18 or 20 days. It is apparent that medium or high amounts of potassium retarded the development of symptoms of systemic infection.

### Discussion

The experimental data indicate that the rapidity with which symptoms of systemic infection develop is not governed primarily by the distance the virus must travel to reach the growing tip. The high-nitrogen plants were much larger than the nitrogen-deficient plants, yet systemic infection appeared in both groups at about the same time. Furthermore, the plants which had received high potassium were about four-fifths the size of those

which had received no potassium, yet systemic infection appeared 9 days later in the high-potassium plants than it did in the potassium-deficient plants.

In all tests, symptoms of systemic infection appeared earlier in plants deficient in nitrogen, phosphorus, or potassium than in plants supplied with the element in question. This difference in the time required for the appearance of systemic infection was most noticeable in the phosphorus and potassium experiments. This variation in time reaction may be due to a physiological process which is probably similar in plants deficient in nitrogen, phosphorus, or potassium. In plants deficient in any of these 3 elements, the element in question is translocated from older mature leaves up to the growing tip. When mature leaves of deficient plants are inoculated, the virus may be translocated from these leaves by this re-utilization process faster than it might be translocated from leaves of a similar age on normal plants. The more rapid development of symptoms of systemic infection brought about by the addition, at time of inoculation, of the element previously deficient does not invalidate this explanation. Several days may elapse before the plant resumes normal growth. Meanwhile, translocation from the mature leaves may still be taking place.

There are several factors which must be considered in an attempt to explain the differences in the time required for the development of symptoms of systemic infection in plants receiving various nutrient solutions. Of these factors, the following are the most important: susceptibility of the inoculated leaf, incubation period of the virus, rate of virus multiplication, rate of movement from the inoculated leaf, rate of movement in the stem, and susceptibility of tissue in the tip. In regard to the first factor, it has previously been shown (3, 4) that plants receiving various nutrient solutions differ in their susceptibility to primary infection. Because of this variation in susceptibility, all plants were so heavily inoculated that many primary lesions developed on all plants. This heavy inoculation was believed sufficient to offset any effect which variations in susceptibility of the inoculated leaf might have on the subsequent development of systemic infection. The experimental evidence does not indicate what other factors might have been affected by host nutrition. The evidence does indicate, however, that nutrition has produced some change in the disease complex, inasmuch as the time required for the systemic development of the disease varied with the nutrient treatment.

It is not yet known how host nutrition affects the systemic spread of the virus. Mineral nutrition may change the rate of protoplasmic streaming, the nature of protoplasmic connections between cells, or the rate of translocation of food materials. Any changes such as these would probably alter the rate of virus spread within the plant.

### Summary

The influence of mineral nutrition on the systemic spread of a yellow strain of tobacco-mosaic virus in tobacco plants in sand cultures was studied by determining the time required for the virus to reach and produce symptoms of secondary infection on tip leaves of inoculated plants.

Symptoms of systemic infection appeared earlier in plants that had received nutrient solutions containing either a deficiency or an excess of nitrogen than in plants that had received a medium amount of nitrogen. It seemed to be immaterial whether the treatment with excess nitrogen started 1 week or 4 weeks prior to time of inoculation.

Plants that had received no phosphorus showed symptoms of systemic infection earlier than plants that had received excess phosphorus for 1 week prior to time of inoculation, and these in turn showed symptoms earlier than plants that had received excess phosphorus for 2, 3, or 4 weeks prior to inoculation. The growth made by the plants was directly correlated with the duration of the excess-phosphorus treatment.

The time of appearance of systemic symptoms was also directly correlated with the duration of the treatment with excess potassium. However, in contrast to the findings in the phosphorus study, the high-potassium treatment retarded growth more than the potassium-deficient treatment.

It is concluded that the systemic development of the disease was accelerated by high-nitrogen nutrition and retarded by either high-phosphorus or high-potassium nutrition. The rapidity with which symptoms of systemic infection developed showed no apparent correlation with the distance the virus had to travel to reach the growing tip.

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# ABSORPTION SPECTRA OF SINGLE CHLOROPLASTS IN LIVING CELLS IN THE REGION FROM 664 $m\mu$ TO 704 $m\mu$ <sup>1</sup>

V. M. ALBERS AND H. V. KNOOR

(WITH TEN FIGURES)

## Introduction

The absorption of light in living plant material, either in the form of living leaves or suspensions of algae, is a complicated optical process. The problems involved have been treated in detail by MESTRE (3). It is well known that the position of the red absorption band of the living material is displaced toward the red end of the spectrum about 20  $m\mu$  from that of the red absorption band of pure chlorophyll  $\alpha$ . WLODEK (5) has suggested that the displacement may be due to an optical property of the living leaf or that it may be due to unstable compounds formed by the pigments in the photosynthetic process.

An attempt has been made by BAAS-BECKING and ROSS (2) to eliminate the complicated scattering processes, usually encountered, by photographing the absorption spectrum of a single *Euglena*, using a microspectrograph. Since they show only a densitometer curve of the spectrogram, and since the resolving power of their experimental arrangement was very low, their results do not give a complete answer to the problem. Furthermore, the *Euglena* cell does not represent a single chloroplast.

This investigation was undertaken for the purpose of determining the absorption spectra of individual chloroplasts, in the region of the red absorption band, with a high resolving power spectrograph, using calibrated plates so that the actual absorption curve in that region could be determined.

## Experimentation

The optical system used for photographing the absorption spectra is shown diagrammatically in figure 1. It consists of a microscope, with pro-

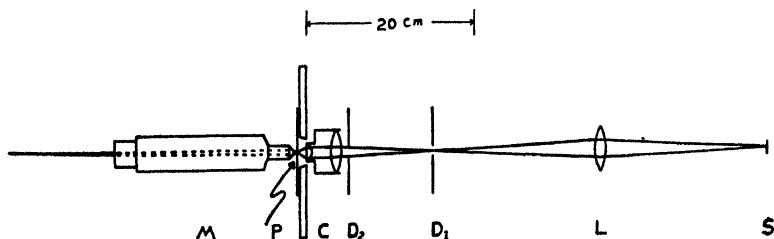


FIG. 1. Optical system used for photographing absorption spectra.

<sup>1</sup> A contribution of the C. F. Kettering Foundation for the study of chlorophyll and photosynthesis.





FIG. 2. Photomicrograph of chloroplasts in *Zygnema*.

vision for critical illumination, and a Hilger E<sub>8</sub> spectrograph for photographing the spectra. The chloroplasts are mounted, either in water or water containing some agar, between a glass coverslip and a glass object slide. This preparation is mounted on the stage of the microscope so that the chloroplasts are in the plane, P. The source of light, S, is a six-volt, 108-watt, ribbon filament lamp. The lens, L, forms a real image of the filament at the diaphragm, D<sub>1</sub>, which is imaged in the plane, P, by the microscope

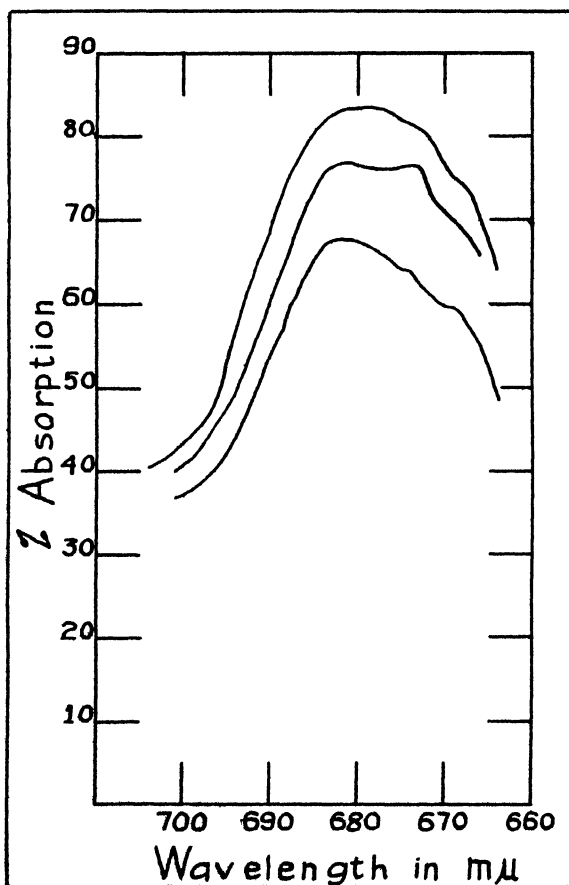


FIG. 3. Absorption spectra of *Protococcus* chloroplasts from one source.

substage condenser, C. The diaphragm,  $D_2$ , is a normal substage condenser diaphragm. With this arrangement,  $D_1$  serves as a field diaphragm, while  $D_2$  serves as an aperture diaphragm. The objective used is a 4 mm. achromat of 0.85 N. A. The microscope is so focused that an image of the chloroplast is formed by the objective on the slit of the spectrograph,  $S_2$ . No ocular is used on the microscope. The diagram (fig. 1) is drawn to scale in all respects except for the distance from the top of the microscope tube to the spectrograph slit. This distance was 50 cm., giving a magnification of 177 diameters. Figure 2 is a photomicrograph of the chloroplasts in a *Zygnema* filament made with a magnification of 200 diameters. The maximum angular aperture of the cone of light from the preparation which can be collected by an objective of 0.85 N. A. is  $116^\circ$ . In this work the aperture diaphragm of the condenser was stopped down considerably below this value, but no means were available for determining the actual angle of the cone used. The field diaphragm,  $D_2$ , is a fixed diaphragm and its diameter is such that a field of

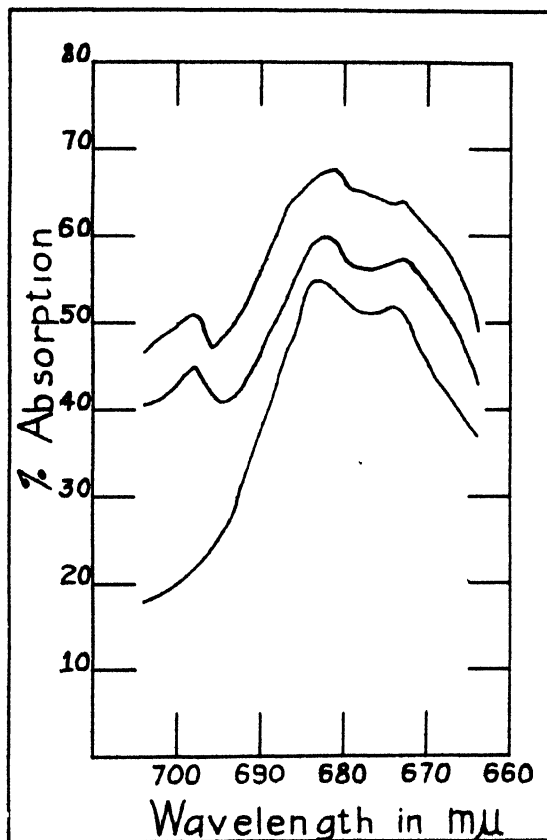


FIG. 4. Absorption spectra of *Protococcus* chloroplasts from another source.

about four times the diameter of the chloroplast is illuminated. Under these conditions, very little light, not actually transmitted by the chloroplast, can be scattered into the spectrograph slit.

In order to determine the percentage of absorption by the chloroplasts as a function of the wavelength, each plate was calibrated. This was done by moving the slide, on the stage, until the image of the chloroplast did not fall on the slit and making a series of exposures on the plate with oscillating neutral screens of known transmission in the light path. The percentage transmission of the chloroplasts at each wavelength was then determined by the method which has been previously described (1). It is not possible to make any correction for reflections occurring at the first surface of the chloroplast.

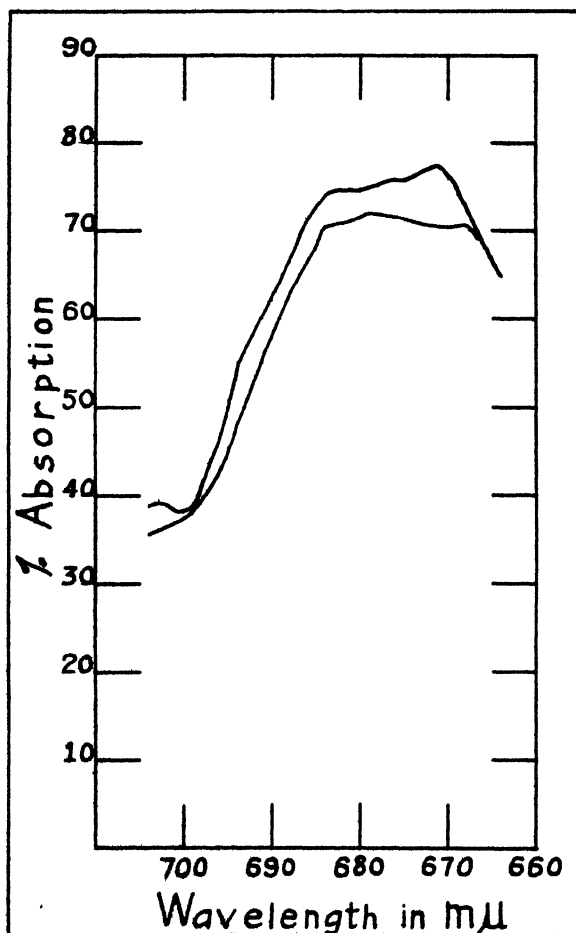


Fig. 5. Absorption spectra of *Protococcus* chloroplasts at a different season.

The sources of chloroplasts used were the algae: *Protococcus*, *Spirogyra*, and *Zygnema*. The spectra were studied only in the region of the red band, i.e., 664 m $\mu$  to 704 m $\mu$ .

### Results

Figures 3 to 6 show the absorption spectra of *Protococcus* chloroplasts. Each absorption curve was made from a different cell. The curves in figure 3 were made from cells gathered from one source and those in figure 4 were made from cells gathered from another source. The two sets of cells were gathered at the same time. The curves in figures 5 and 6 were made from cells which were gathered at the same time and place. They were gathered at a different time of year than those from which the curves in figures 3

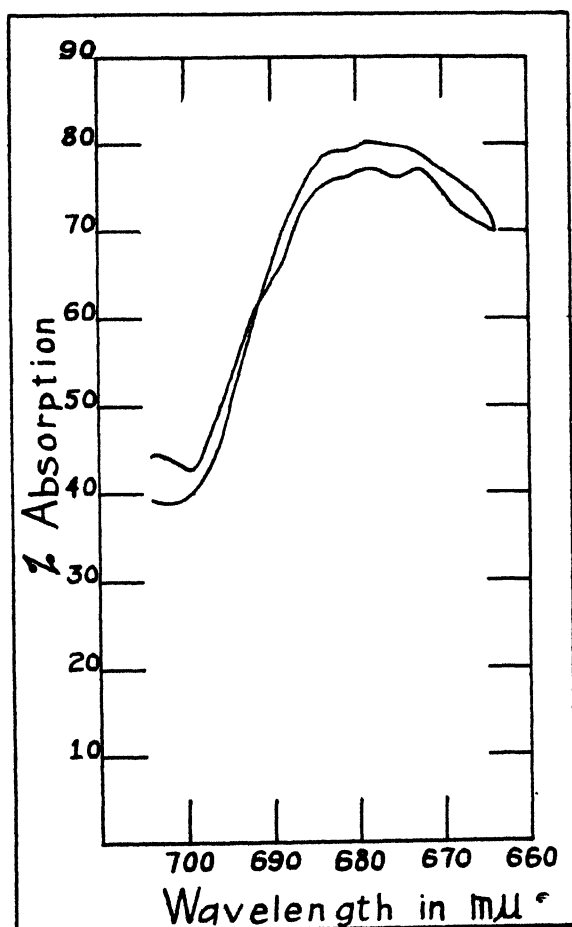


FIG. 6. Absorption spectra of *Protococcus* chloroplasts, same time and place as in figure 5.

and 4 were made. Figures 7, 8, and 9 show absorption curves of the chloroplasts of *Spirogyra*. All of the cells used were gathered from the same source and at the same time. Figure 10 shows absorption curves of the chloroplasts of *Zygnema*. In this case, the chloroplasts were all from cells on the same filament. In all cases, the algae were gathered immediately before the spectra were photographed to insure a minimum of danger of injury to the cells before they were used.

The striking characteristic of these absorption curves is the presence in nearly all cases of several maxima of absorption, rather than a single maximum at about 680 m $\mu$ , as ordinarily reported. The relative intensities of these maxima vary from one cell to another, indicating that they are produced by different substances. Table I gives the wavelength readings of the maxima from all of the curves.

In the case of the *Zygnema* chloroplasts, the individual maxima are not as well resolved, although the shapes of the curves indicate that the same bands are present.

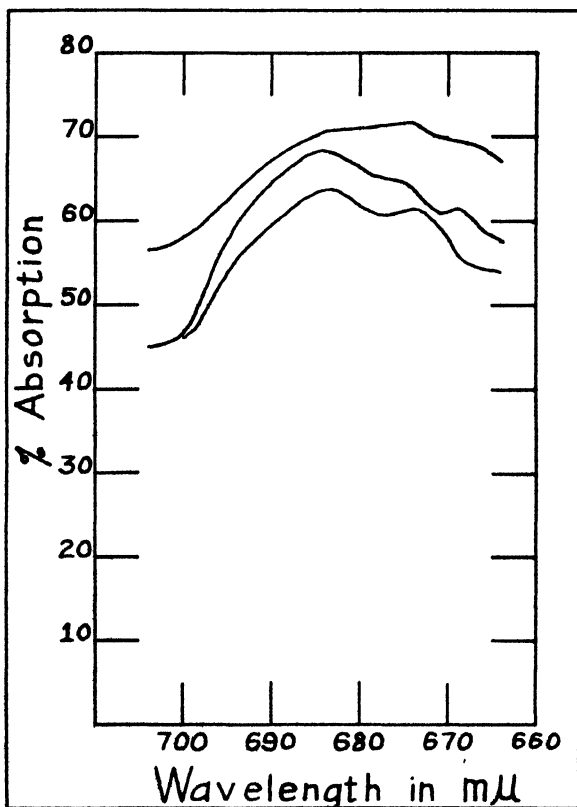


FIG. 7. Absorption spectra of *Spirogyra* chloroplasts.

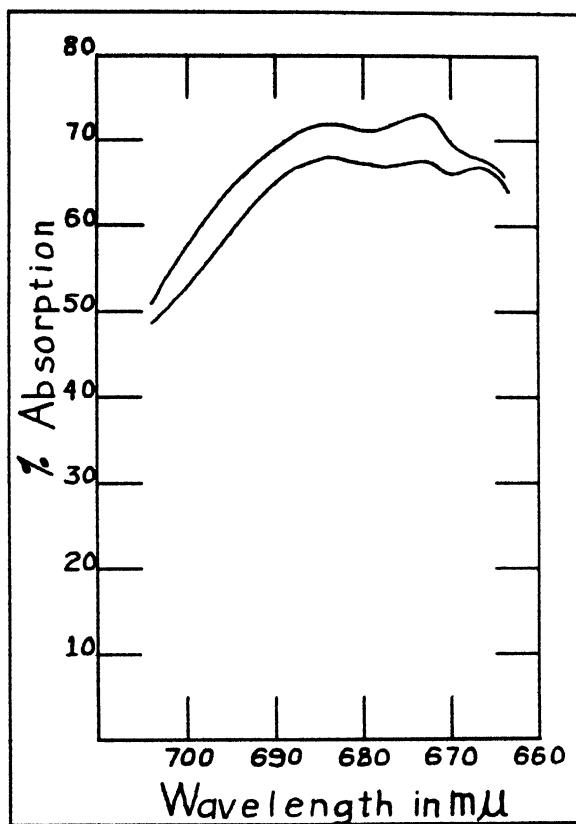


FIG. 8. Absorption spectra of *Spirogyra* chloroplasts, same time and place as in figure 7.

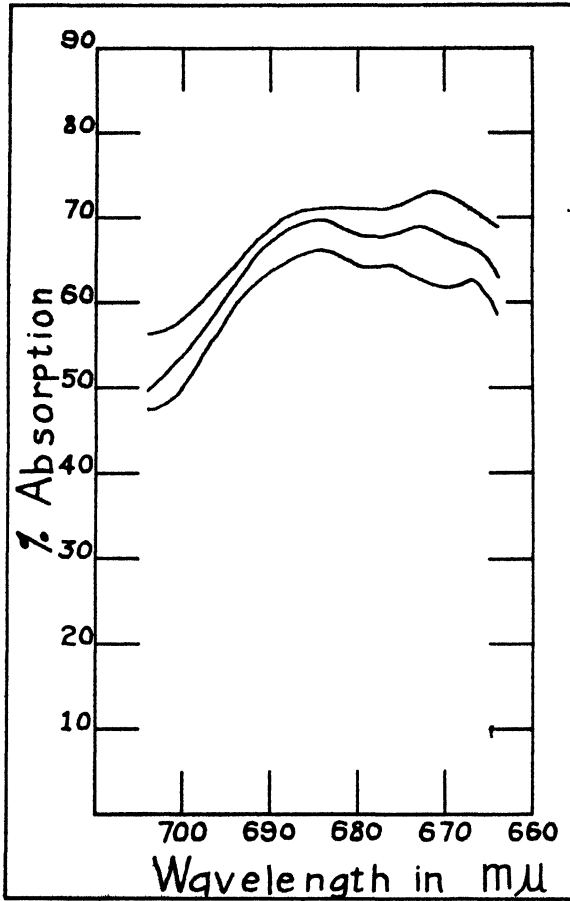


FIG. 9. Absorption spectra of *Spirogyra* chloroplasts, same time and place as in figure 7.

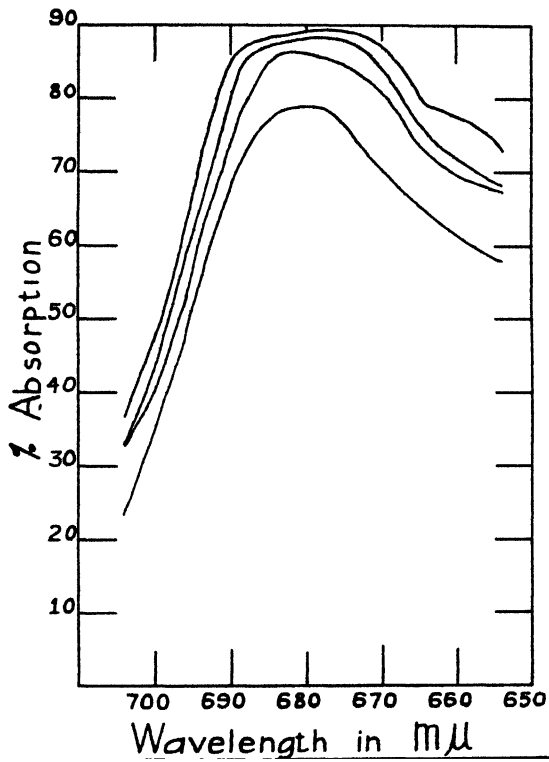


FIG. 10. Absorption spectra of *zygneema* chloroplasts, all from cells of the same filament.



TABLE I

SPECIES OF ALGAE	WAVELENGTHS OF BANDS IN M $\mu$			
<i>Protococcus</i> (1).....	698	687	681	673
<i>Protococcus</i> (2).....	698		682	673
<i>Protococcus</i> (3).....		687	683	674
<i>Protococcus</i> (4).....			←680→	673 669
<i>Protococcus</i> (5).....			682	674
<i>Protococcus</i> (6).....			682	674 669
<i>Protococcus</i> (7).....			684	679
<i>Protococcus</i> (8).....			683	678 673
<i>Protococcus</i> (9).....			683	676 671
<i>Protococcus</i> (10).....			684	679 668
<i>Spirogyra</i> (1).....			684	673
<i>Spirogyra</i> (2).....			684	673 667
<i>Spirogyra</i> (3).....			684	676 667
<i>Spirogyra</i> (4).....			684	673 667
<i>Spirogyra</i> (5).....			682	672
<i>Spirogyra</i> (6).....			683	674
<i>Spirogyra</i> (7).....			684	675 669
<i>Spirogyra</i> (8).....			683	674
<i>Zygnema</i> (1).....			←680→	
<i>Zygnema</i> (2).....			683	675
<i>Zygnema</i> (3).....		←688→		675
<i>Zygnema</i> (4).....		←687→		675

### Discussion

The readings of the positions of maxima on these curves probably do not, in all cases, represent the positions of the maxima of the individual bands. The bands occur with so little wavelength separation, compared to their width, that one maximum on a curve may be the resultant of two adjacent bands, and the wavelength, read for the resultant, would then be influenced by the relative intensities of the two bands. This is probably true both in the case of the maximum appearing at about 673 m $\mu$  and the one at about 683 m $\mu$ .

In the spectrum of solutions of the two green plant pigments, chlorophylls  $\alpha$  and  $\beta$ , each has a single sharp maximum at 661 m $\mu$  and 643 m $\mu$  respectively. The band in living leaves occurring at 680 m $\mu$  has always been attributed to the chlorophyll  $\alpha$  in the leaf. The results of this investigation indicate that there are either other pigments present, giving absorption comparable to that due to chlorophyll  $\alpha$ , or that chlorophyll  $\alpha$  enters into the photochemical reactions in photosynthesis in several steps, forming reaction-products having absorption bands at slightly different wavelengths. This last assumption seems more reasonable than the first, since one would expect that other pigments, having absorption comparable to that of chloro-

phyll  $\alpha$ , would have been isolated by chemists working with the leaf pigments. These results also indicate quite definitely that the wavelength difference between the absorption band, as observed in pure chlorophyll  $\alpha$  solutions and the living leaf, is not produced by the optical properties of the leaf tissue.

WILLSTÄTTER and STOLL (4) have pointed out that the increased light path in the albino leaf results in an increase in absorption by its tissues, giving a false notion of the actual amount of absorption by the pigments. By the same argument, it is evident that, in a given leaf, the increased path of the light of wavelengths where the absorption of the pigments is less, will result in a corresponding increase in the absorption in the tissues for these wavelengths, thus tending to smooth out the absorption curves. This might explain the fact that all observers working with the entire leaf have observed only a single broad absorption band at 680 m $\mu$  instead of several maxima. This idea is still further supported by the fact that the *Zygnema* chloroplasts, which were the largest chloroplasts studied, show the least resolution of the band maxima.

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## ON THE DETERMINATION OF PLANT ENZYMES<sup>1</sup>

Z. I. KERTESZ

The fact that plant life depends a great deal on enzymes was recognized early, and enzymes are receiving the constant attention of workers in botanical sciences. Because one deals with enzyme *action* rather than with isolated enzymes, enzyme chemistry is chiefly a science of methods. The results obtained in any specific case may yield a basis for conflicting conclusions. The reason for this lies in the fact that most methods for the estimation of enzymes have been worked out by enzyme chemists rather than by those interested in enzyme chemistry as a tool for the botanical sciences. The methods to be used, and the facts observed will greatly depend on the point of view of the worker. In too many cases, results and conclusions obtained by the botanist by the use of strictly enzyme chemical methods fail to take into account the actual rôle and significance of the enzyme in the plant. The enzyme chemist is aware of the unstable character of the enzyme. The botanist has to go one step farther because he is dealing with the equally unstable and complex plants which produce and contain the enzyme.

From the standpoint of the determination of plant enzymes, the conditions under which the substrates of enzyme action exist in the cell should be considered first. The protoplasm is a constantly changing system in which the different materials exert great influence on each other. It has also been shown that many important chemical constituents of the cell such as proteins, polysaccharides, and lipids may form homogeneous or heterogeneous complexes or aggregates which have different properties, hence behave entirely differently toward enzymes. The size and complexity of such aggregates, furthermore, are undergoing almost steady changes in the protoplasm. Naturally the substrate may also react with other chemical constituents of the cell, producing reversible or irreversible compounds of different affinity to the enzyme. Thus the condition of the substrate of enzyme action in the cell may be entirely different from that in a test-tube, and also may show great qualitative and quantitative variation within a short period of time.

Similar to an enzyme, a substrate may also go through changes making it unavailable or "inactive." The substrate may be separated from the enzyme by occlusion. Occlusion, however, should be regarded as a case of spatial

<sup>1</sup> Read before the joint session of the American Society of Plant Physiologists, American Society for Horticultural Science, and the Physiological Section of the Botanical Society of America with the A. A. A. S., held at Rochester, New York, June 17, 1936. Also an introduction to the recommendations on the determination of plant enzymes contemplated by the Committee on Chemical Methods of the American Society of Plant Physiologists.

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separation. The substrate may be separated from the enzyme by adsorption or by membranes within the cell. Because of its great importance, spatial separation will be discussed in detail later in this paper.

But let us turn to the enzymes and consider the factors which may influence their activity in the cell. We know little about the mechanism of enzyme formation. In many cases, especially in microorganisms, the cell is capable of producing new enzymes to suit changed living conditions; and it is well known that the activity or quantity of the enzyme can be greatly influenced. The much debated existence of "zymogenes" or "proenzymes" is of historical interest only. It has been shown for practically every case that where the presence of a zymogene was contemplated, upon changing the conditions of determination and especially upon the addition of specific activators, the full activity of the enzyme could be attained. Thus the predecessor of the active enzyme is not a zymogene in the old sense of the word but the enzyme in an insoluble form (amylases), or lacking certain chemical changes (or activators) to become active (papain). Indeed, sometimes enzymes are formed from inactive compounds by heat or by the action of other enzymes (5).

A very interesting case of this sort is the germination of seeds. It is most probable that in the first stages of germination there are no new enzymes formed or set free from a "zymogene." The increased enzyme activity seems to be caused by the liberation of strongly adsorbed enzymes. The proteolytic activity in the seed commences as soon as the water is taken up. The liberation of the amylases, on the other hand, is apparently the result of the action of proteolytic enzymes. CHRZASZCZ and JANICKI (2) established the fact that when dormant wheat, barley, rye, and other cereal seeds are treated with proteolytic enzymes, there is a great increase in the amount of amylase which passes into solution. The amylase was present in the dormant cell with all three functions (liquefaction, dextrinization, saccharification) but is inactivated by being adsorbed on a *sisto-material* (3). Later during the germination, however, there is a demonstrable formation of new enzymes. This observation shows how critical and careful one must be with the results of enzyme determinations, and in accepting information available in the literature.

Thus enzymes may be present in plants without exerting any activity. Starch is present in plants containing active amylases but is not hydrolyzed while the cell structure is intact. The presence of inactive enzymes in the cell, apparently very near to the substrates of their action, is another example of the marvelous coordination within the cell.

The inactivity of the enzyme in the cell may be caused by the absence of activators, spatial separation, formation of inactive compounds, and perhaps by a number of other factors. Undoubtedly the case of spatial separation is the most frequent factor and of most importance in controlling enzyme

action *in vivo*. There are two classes of conditions which may cause inactivity of the enzymes by spatial separation. One is occlusion, or a selective adsorption of the enzyme or the substrate on other cell constituents. In this class also belong the cases in which both the enzyme and substrate are adsorbed by the same material, and the enzyme exerts no activity. The complexity of enzyme relations in the cell is well indicated by the observation that in certain cases, when the enzyme and its substrate are adsorbed, the velocity of the enzyme reaction is not altered at all, while in other cases it is completely nullified. Yeast saccharase loses none of its activity when adsorbed by a variety of solids (8, 9); on the other hand, amylase is inactive while adsorbed (by the "sisto-material") in ripening and dormant cereals (3). Our knowledge of surface reactions must make further advances before we will be justified in doing much speculation on what the mechanism of such reactions is.

The other and probably more common example of spatial separation is that in which the enzyme and substrate are separated by gel-like or other colloidal membranes. Such membranes have been described many times and appear to be common in the cell as one of the chief means of maintaining the organization therein. It has also been observed that protoplasmic granules are often surrounded by a lipoid or fatty film which protects them from the action of proteolytic enzymes. Often upon removal of the fat or lipoid the granules are readily digested by the enzymes (1). In the presence of such colloidal membranes the velocity of the reaction will naturally depend on diffusion, the rate of which will be determined by the permeability of the membrane. Undoubtedly selective permeability plays an important rôle in such cases.

Naturally a combination of both classes of conditions may also be the reason for the inactivity of the enzyme in the tissue. With our present knowledge it is often impossible to say whether the inactivity is caused by adsorption or by the separation of the enzyme and its substrates by membranes.

To obtain enzyme solutions from plants, the natural structure of the cell must be destroyed. It was observed long ago that enzyme action in the cell is quite different from the action of the same enzyme after injury or destruction of the cell structure. The action of enzymes in the protoplasm is regulated by different means; but this coordination characteristic of the living cell is disturbed by injury. It is well known that upon destruction of the cell structure by drying, freezing, or by exposing it to the effect of antiseptics the enzyme activity in the plant tissue increases as a result of the disorganization of the cell. This is mostly caused by the fact that, by the destruction of the cell structure, the enzyme gets into more intimate contact with its substrate. Thus by a destruction of the gel-like membranes in the protoplasm,

the rate of the reaction will no longer depend on the rate of diffusion. Similarly other cases of spatial separation are terminated by a destruction of the cell structure.

There are other changes which may come about when the contents of a cell or cells are mixed. It has been shown that in such cases enzymes normally present in the cell, but not in contact, may exert considerable digesting effect upon each other. We have no evidence that all enzymes are proteins but it is safe to say that many of them are. These enzyme proteins may be digested by proteolytic enzymes, causing loss and even nullification of the enzyme activity. This often happens during autolysis of plant extracts.

It was observed some twenty years ago that hydrogen sulphide activates papain. Later work revealed that compounds containing the  $-SH$  group, as certain thiol compounds and glutathion, have very beneficial influence on enzyme action. The apparent rôle of glutathion is the reversal of partly oxidized papain into the reduced form (4). We do not have any information regarding the necessity of glutathion for the action of papain in the cell. On the other hand all papain preparations can be activated by glutathion, showing that upon mixing the cell contents, or during preparation, a certain reversible oxidation of the enzyme occurs. Strongly reducing thiol compounds, as glutathion, are not rare in nature. Occasionally when the cell structure is destroyed, these compounds find access to certain enzymes and activate them. Many cases of activation by chemical constituents of the cell are also known. It is most likely that, in many cases, natural constituents of the cell have marked influence on the activities of certain enzymes. This appears to be again one way by which the coordination of enzyme action in the protoplasm is governed. Our knowledge of enzyme activators is rapidly increasing (11) and much work is being conducted to explain this interesting phenomenon.

One basic difference between plant chemistry and the study of plant enzymes is that for enzyme work the plant material cannot be preserved. The determinations of plant enzymes must be performed on fresh material because the extremely sensitive and labile enzymes are much influenced or even destroyed by drying, heat, alcohol, and other physical and chemical treatments. The only known method to preserve plant material for enzyme work is to freeze it at very low temperatures, and store it at those temperatures, under conditions preventing desiccation. This method is far from being entirely safe, but can be used in emergency and for short periods of time.

The enzyme in plant materials is often determined in the press sap or in extracts. Because of the limited solubility of many enzymes, much care should be exercised in such cases to obtain reliable results. WILLSTÄTTER, for instance, found eight individual amylases in leucocytes, but only four

of them were easily soluble in water; the others appeared to be bound to cell constituents (12). Another interesting case in this line is that of zymase. Although LEBEDEW and others long ago described the preparation of cell-free extracts from yeasts, which were able to produce alcoholic fermentation, many workers failed to obtain such active extracts. It was not until recently that NILSSON and ALM (10), in VON EULER's laboratory, made a study of this problem and established the conditions by which the zymase, bound to other cell constituents, can be rendered water soluble. Thus by making an enzymatic analysis of the extract from plants, one is hardly justified in drawing conclusions about the presence and activity of the enzymes in the cell. Attention is called again to the fact that, for a successful extraction of the enzymes, the structure of the cell has to be destroyed by drying, freezing, autolysis, or chemical means all of which may alter the activity of the enzyme for reasons outlined previously. In many cases, working with suspensions of the ground tissue is satisfactory if the enzyme is not easily soluble in water or glycerol.

As far as the quantitative determination of the enzyme activity in the cell sap is concerned, in certain cases conclusions may be drawn from such results as regards the activity of the enzyme *in vivo*. The sap should be expressed at low temperatures, and with pressures not over 6000–8000 atmospheres because higher pressures cause partial or complete inactivation of the enzymes (7). The limit of safe pressure has been found to be astonishingly similar for different enzymes.

After having an enzyme extract or a tissue suspension ready for the determination of its enzyme content, a number of questions arise. Should the determination be performed at the natural hydrogen ion concentration of the plant, or at that of the cell sap (if known), or should it be adjusted to the optimum of the enzyme action? What concentrations should be used? What should be done about activation or removal of natural inhibitors? What about temperature, antiseptics, etc.? With the raising of all these questions we arrive at the main point in the present discussion.

The botanist needs the help of the enzyme chemist. Every one of the above questions has to be answered. The answer in most cases cannot be generalized because the factors influencing different enzymes and the conditions insuring their maximum activity vary. It is the function of the enzyme chemist to establish these facts. Having this essential information available, the botanist should proceed to determine under optimal conditions the activity of the enzyme present. Then he should proceed to obtain information on the rôle and significance of the enzyme in the plant. He ought to investigate the enzyme action under as nearly natural conditions as possible.

The most satisfactory way of studying enzymes would be to observe their action in the undisturbed cell. While there is some hope that this may be



accomplished in the future, there are only a few attempts recorded in the literature reporting even moderate success. Ideal experimental conditions for the determination of plant enzymes would be reached by the addition of an infinitesimal quantity of the substrate to the cell, followed by an immediate determination of the enzyme action during an extremely short period of time. The difficulties in performing such a determination are obvious, and we are restricted at the present to an estimation of the quantity of the enzyme present in the tissue. The best approach to ideal conditions suggested thus far for this purpose is the enzymatic microtechnic originated by LINDERSTRØM-LANG and coworkers (6). This method is suitable for the determination of enzymes in minute quantities of tissues or extracts and much valuable information has been obtained already on the distribution of enzymes in animal and plant tissues. But here again we obtain information on the possible maximum activity of the enzyme only, and one should exercise great care in drawing conclusions in regard to the actual normal activity of the enzyme in the cell.

Unfortunate as it is, we do not have any dependable method for measuring enzyme activity *in vivo*. The analysis of plant material for different substrates and reaction products of enzyme action by micro-methods is of use sometimes, but the lability of the composition of the cell, and the many different reactions proceeding may entirely mask the changes in the substrate or the appearance of reaction products of the investigated enzyme reaction.

In the foregoing discussion an attempt has been made to show some of the vital points which have to be considered in connection with the determination of plant enzymes. With the application of the methods of physical chemistry, our knowledge of the protoplasm is rapidly increasing; but still more progress is needed before we can be certain of the condition of the enzymes in the cell. Our knowledge of enzymes, on the other hand, is advancing rapidly, and we can be hopeful of important gains in the not too distant future. While we know enough of the factors which have to be considered in connection with enzyme studies on plants, yet we are not able to make studies on the plant cell in its natural condition. Realizing the need for such methods is the first step toward attaining them.

Meanwhile it is of utmost importance that all work on plant enzymes be made by dependable methods, having clearly in mind all known factors which do or may influence enzyme action. But of even more importance is it that great care should be exercised in drawing conclusions from results obtained *in vitro* regarding the natural condition, activity, and rôle of enzymes within the plant cell.

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# INCREASE OF TRANSPIRATION RATES OF TOMATO LEAVES DUE TO COPPER SPRAYS<sup>1</sup>

KENNETH K. KRAUSCHE AND BASIL E. GILBERT

Several observers have reported that leaf transpiration is increased with many plants by the presence of copper sprays. It has also been quite definitely proven, especially by WILSON and RUNNELS (4) with *Coleus*, that the greatest increase takes place at night. Little data, however, have been gathered to explain this phenomenon. This paper reports certain experiments carried out with tomato plants which both substantiate the findings of earlier workers as to the increase of transpiration rates due to copper sprays and give some evidence as to the mechanism by which it takes place.

## I. Effect of copper sprays on transpiration

Tomato seedlings were transplanted to 5-inch flower pots which had been made impervious to moisture by treatment with paraffin. Each pot contained the same weight of soil of known moisture content.

When the plants had reached a height of 7 to 10 inches, a disc of water-proofed paper, with a hole in the center for the stem of the plant, was paraffined over the top of the pot. Cotton was used around the base of the stem as a plug for the hole. The plug was removed when it was necessary to add more water to the soil.

The transpiration rate of each plant during the day and night was determined as follows: The soil moisture was made up to the desired percentage, and the plants were set on a bench in the greenhouse. Weighings were made at 6:30 A.M. and at 6:30 P.M. The loss in weight was the amount of water transpired during the previous 12-hour period. At each weighing, enough water was carefully added through a pipette to bring the weight back to the original.

After the transpiration rates had been established, the plants were paired, each pair made up of two plants showing approximately equal water loss. One plant of each pair was called the test plant (T), and the other the control plant (C). In some cases, three plants were grouped together—each of two test plants being checked against the same control. The ratios T:C for day and for night were calculated. Then the test plants were sprayed at the beginning of the day period with the desired spray, and the control plants were sprayed with water. The transpiration rates of all the plants were observed during that day and during the following night. The day ratios and the night ratios of T:C were again calculated. The ratios T:C after

<sup>1</sup> Published by permission of the Director of Research as Contribution no. 505 of the Rhode Island Agricultural Experiment Station, Kingston, Rhode Island.

spraying were then computed on the basis of making T:C for the corresponding period before spraying equal to unity. The change in ratio gave a measure of the effect of the spray on transpiration. This procedure is similar to that instituted by DUGGAR and COOLEY (2) and used since then by several other investigators.

The Bordeaux formula usually recommended for early blight on tomatoes is 4-4-50, while the strength of Burgundy spray advocated is usually 1.5-1.5-50. It will be noted that the formulae used in this investigation are much stronger than these. They were purposely chosen, after preliminary tests, in order to insure a measurable amount of visual spray injury. The lime used in making the Bordeaux was slaked lime and contained 68 per cent. calcium oxide. Burgundy spray was prepared by mixing 1 pound of copper sulphate and 2 pounds of sodium carbonate in 50 gallons of solution. Household washing soda was used as the sodium carbonate.

Each spray was tested for excess available copper with a 10 per cent. potassium ferrocyanide solution. Twenty cc. of the spray solution were put in a beaker and a few drops of potassium ferrocyanide solution were added. A red-brown precipitate indicated the presence of available copper in the spray. A negative test indicated that all the copper was in a combined and insoluble form. The Bordeaux 8-8-50 gave a negative copper test. The Bordeaux 8-4-50 gave a decidedly positive test and the 8-12-50 a negative test. The Burgundy 8-8-50 contained a great excess of available copper.

In the first series, three trials were made, each using sixteen plants. Four different sprays were employed: Bordeaux 8-8-50, 8-4-50, 8-12-50, and Burgundy 8-8-50. The soil moisture was kept at 15 per cent. The values of the ratio T:C of the sprayed plants (when T:C of the control plants equals 1.00) are tabulated in table I.

TABLE I  
EFFECT OF CERTAIN COPPER SPRAYS ON TRANSPIRATION OF POTTED TOMATO PLANTS  
AS SHOWN BY THE RATIO OF THE TEST PLANTS TO CONTROL  
PLANTS AFTER SPRAYING THE FORMER

SPRAY USED	T: C AFTER SPRAYING	
	DAY	NIGHT
Bordeaux 8-8-50 .....	0.87	1.52
Bordeaux 8-12-50 .....	0.91	1.54
Bordeaux 8-4-50 .....	0.91	1.72
Burgundy 8-8-50 .....	1.18	2.54

Similar trials were made to determine the effect of the spray film applied to the upper surfaces of the leaves only, and to the lower surfaces only. In applying the spray to a plant, the plant was held in one hand either facing

directly into or directly away from the spray stream as it issued from the nozzle. By careful manipulation, a good coverage could be obtained on either surface alone. The spray used was Bordeaux 8-8-50. The soil was kept at 15 per cent. soil moisture. In table II the results are summarized.

TABLE II

EFFECT OF METHODS OF APPLICATION OF BORDEAUX 8-8-50 ON TRANSPIRATION OF POTTED TOMATO PLANTS AS SHOWN BY THE RATIO OF TEST PLANTS TO CONTROL PLANTS AFTER SPRAYING THE FORMER

LEAVES SPRAYED	T: C AFTER SPRAYING	
	DAY	NIGHT
On both surfaces .....	0.87	1.52
On upper surface .....	1.01	1.30
On lower surface .....	0.92	1.18

Table III shows the results of spraying potted plants growing in saturated soil (30 per cent. soil moisture) compared with the sprayed plants growing in 15 per cent. soil moisture. The spray used was Bordeaux 8-8-50.

TABLE III

EFFECT OF BORDEAUX 8-8-50 ON TRANSPIRATION OF POTTED TOMATO PLANTS WITH DIFFERENT SOIL MOISTURES AS SHOWN BY THE RATIO OF TEST PLANTS TO CONTROL PLANTS AFTER SPRAYING THE FORMER

SOIL MOISTURE	T: C AFTER SPRAYING	
	DAY	NIGHT
%		
15 .....	0.87	1.52
30 .....	0.66	1.86

In all these tables, the data are recorded separately for day and for night because of the increased significance which this method lends to the figures. It should be stated, however, that in all cases the total transpiration for the 24-hour period was increased by spraying, from a few per cent. to 105 per cent. There was considerable variation among the plants receiving the same treatment but the trends were consistent in all cases.

At the conclusion of these runs, some of the plants were allowed to remain on the bench without the addition of water. In every case, the sprayed plants wilted perceptibly before the control plants were affected.

The results of experiments on transpiration agree with those of WILSON and RUNNELS (4) and those of other investigators in that they show that spraying with Bordeaux and Burgundy increases the transpiration of tomato plants and that the greatest effect is at night.

The greatest effect was brought about by those sprays which were very likely to cause injury. This is shown by data in table IV which resulted

**TABLE IV**  
**AMOUNT OF SPRAY INJURY IN THE FIELD ASSOCIATED WITH DIFFERENT COPPER SPRAYS**

GROUP*	PERCENTAGE OF LEAFLETS INJURED			
	BURGUNDY 8-8-50	BORDEAUX 8-4-50	BORDEAUX 8-8-50	BORDEAUX 8-12-50
	%	%	%	%
I .....	85.0	14.0	1.7	3.5
IV .....	36.0	0.8	1.0	0.0
III .....	28.5	0.2	0.0	0.2
VI .....	4.5	0.0	0.0	0.0
V .....	7.0	0.8	0.0	0.0
VII .....	5.2	0.2	0.0	0.0
II .....	7.5	0.0	0.0	0.0
IX .....	2.5	0.0	0.0	0.0
VIII .....	4.3	0.0	0.0	0.0

\* These groups illustrate varying climatic conditions.

from a field study of the effect of environmental conditions upon spray injury. Burgundy 8-8-50 produced greater increases in both transpiration rates and spray injury than the other sprays used. It perhaps might be expected that severely injured foliage would lose water more rapidly than foliage only slightly injured. It is thus also possible to conceive of the increased transpiration rate as being associated with spray injury.

## II. Microscopical and microchemical investigations

There being little doubt that copper sprays bring about increased transpiration and spray injury under certain conditions, the mechanism of this phenomenon is of great interest. It seemed desirable to study stomatal behavior in order to determine any relationship which may exist with increased transpiration, especially since WILSON and RUNNELS (4) have suggested that "the accelerating influence of a Bordeaux film on the transpiration rate of a plant is due chiefly to a change in the rate of cuticular transpiration."

Stomatal counts were made on the upper surface and on the lower surface of the leaves using the Leitz ultrapak in conjunction with a Spencer research microscope. The Leitz ultrapak is an attachment which focuses a beam of light, from an illuminator, on the upper surface of the object at the focal point of the microscope. Thus, the leaves could be observed in their natural state while still attached to the plant.

Potted tomato plants about 10 inches in height were used. Pots were set on their sides and leaves held in place on the stage by strips of paper. Both large and small leaves of several plants were used. A total of 100 fields were counted on the lower surface and an equal number on the upper surfaces. Table V shows the results of the count. There were 61.6 times as many stomata per unit area on the lower surface as on the upper surface.

TABLE V  
STOMATAL COUNTS MADE ON THE FOLIAGE OF POTTED TOMATO PLANTS

SURFACE	AVERAGE PER FIELD	AREA OF FIELD	THOUSANDS PER SQUARE INCH
		<i>sq. in.</i>	
Upper .....	0.123	0.0000873	1.41
Lower .....	7.580	0.0000873	86.83

Although the number of stomata per unit area on the lower surface was 61.6 times as great as on the upper surface, no difference of such great magnitude existed between the change in transpiration when leaves were sprayed on the lower surface and when they were sprayed on the upper surface. In fact, where the stomata are more numerous, the spray film on the lower surface significantly depressed transpiration during the day.

A series of observations was made with the same apparatus, in order to observe directly the effect of sprays on stomata. Some of the plants were sprayed with Bordeaux 8-8-50, while others were untreated. The spray was applied in the morning and observations were made at 3- to 6-hour intervals for more than 24 hours. During this period the soil moisture of both sprayed and check plants was kept at 15 per cent.

Observations on the sprayed leaves were made either directly through a thin film of spray or by flaking off a particle of spray and immediately observing the uncovered stomata.

In observing the stomata, it was found that their general appearance varied very little at any time. The average stoma appeared elongated, with a narrow slit-like stomatal pore about  $15\ \mu$  long which never opened very widely, if at all. Occasionally one was found which was shorter and much wider than the rest. If any visible opening existed between the guard cells, the stomata were called open; if the pore was found to be definitely closed, it was recorded as closed. There were many stomata, however, which even after careful and patient study could neither be called definitely open or closed because of a blurred appearance in the stomatal pore or because of other reasons. These were recorded as doubtful. The percentage of the total number of stomata in the several conditions was computed for each observation. The summary of the study appears in table VI.



**TABLE VI**  
**CONDITION OF STOMATA OF SPRAYED AND UNSPRAYED POTTED TOMATO PLANTS**  
**DIRECTLY OBSERVED WITH A LEITZ ULTRAPAK**

TIME	UNSPRAYED			SPRAYED		
	OPEN	CLOSED	DOUBTFUL	OPEN	CLOSED	DOUBTFUL
11: 30 A.M. ....	73.7	12.4	13.9	69.5	9.3	21.2
2: 30 P.M. ....	80.9	9.5	9.6	79.3	6.5	14.2
5: 30 P.M. ....	14.5	85.5	0.0	16.5	74.7	8.8
8: 30 P.M. ....	22.6	71.7	5.7	23.0	72.3	4.7
11: 30 P.M. ....	20.2	69.0	10.8	18.7	69.2	12.1
5: 30 A.M. ....	46.8	42.6	10.6	44.0	42.0	14.0
9: 30 A.M. ....	59.4	26.5	14.1	57.9	26.5	15.6
1: 00 P.M. ....	70.0	27.0	3.0	64.5	30.5	5.0

It is safe to say that the general appearance of the stomata of sprayed tomato leaves and those of the unsprayed tomato leaves were the same, except that some of the stomatal pores of the former appeared to be clogged or sealed by minute particles of dried spray.

Spots of spray injury on the leaves were examined around the margin, where the injury had not entirely destroyed cellular structure, in a search for evidence of a relationship between stomatal behavior and spray burn. The burn appeared to involve the ordinary epidermal cells and stomata indiscriminately.

It was indicated by the results of the preceding work that the effect of copper sprays on tomato plants is not primarily a phenomenon of the stomata. Attention was then focused on the epidermis of the foliage and microchemical tests were made in order to determine its chemical nature.

Two series of tests were performed: one on the leaves of potted tomato plants 1 foot high and grown in the greenhouse; and the other on the foliage of mature plants grown in the field. Freehand sections were made and tested with standard microchemical reagents.

Cross sections of the tomato leaf were placed on a glass slide. A few drops of concentrated sulphuric acid were allowed to run onto a section while it was being observed. The action of the acid on the epidermis was noted.

Other sections were saturated with iodine-potassium iodide reagent and any color changes noted. Then 75 per cent. sulphuric acid was allowed to flow over the section and further color changes were observed.

A third test consisted of wetting fresh sections with a solution of chlor-iodide of zinc and observing the color reaction in the epidermis.

The last test consisted of placing the sections on a glass slide in contact with a solution of Sudan III. The epidermis was examined for evidence of the absorption of the dye.

The results of the two series were the same. The epidermis was immediately soluble in concentrated sulphuric acid. With the iodine-potassium iodide reagent the epidermis was not colored, but upon the addition of 75 per cent. sulphuric acid the epidermis became a deep blue. With chloriodide of zinc, the epidermis assumed a violet hue. Sudan III did not color the epidermis. From these tests it was concluded that the epidermis was composed of undifferentiated cellulose; that is, cellulose which had not become lignified, cutinized, or suberized.

Since all the phases of the work on stomata led to the same conclusion, that they are not the mechanism through which copper sprays increase transpiration, then the action must take place directly through the epidermis. If the epidermis be composed of undifferentiated cellulose or of lignified cellulose it is permeable, while cutinized and suberized cell walls are not, as indicated by STILES (3). Since it was ascertained that the epidermis of the tomato plants used in the experiments was composed of undifferentiated cellulose, they were permeable. The presence of a permeable epidermis allows a reasonable explanation of the passage of water outward from the leaf, without involving the stomata, by the process of exosmosis caused by the presence of the spray film on the surface.

### Conclusions

It is probable that the soluble copper and calcium on a leaf surface, so readily permeable as a cellulose membrane is thought to be, will readily penetrate to bring about changes in permeability in the membranes of the guard and mesophyll cells so that water loss takes place at varying rates. That copper does change the permeability of cell membranes has been shown by COOK (1) with *Nitella*.

From earlier work, substantiated by the experiments outlined in this paper, it seems evident that copper sprays increase transpiration particularly at night.

Furthermore, evidence is given to show that with tomato leaves the major portion of the increased transpiration is not stomatal and that copper sprays do not seem to affect the behavior of stomata.

Transpiration with the tomato must take place largely through the cuticle and this must be subject to great modifications in rate by copper sprays.

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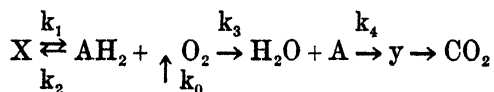
# PROOF OF THE FLUX EQUILIBRIUM RELATION<sup>1</sup>

GORDON MARSH

(WITH THREE FIGURES)

## Introduction

A quantitative connection has been derived (3) between the measured E.M.F. of a living cell or tissue possessing electrical polarity and the velocity of cell oxidation, following LUND'S (1) oxidation-reduction theory of bioelectric currents. The equations provide a consistent description of the major facts of continuous bioelectric currents and respiratory exchange. The respiratory process was schematized as follows:



where X is the precursor of the reductant, AH<sub>2</sub>, oxygen is supplied by diffusion from the external medium, and the oxidant, A, is destroyed through further transformation. k<sub>1</sub>, etc., are reaction velocity constants, k<sub>0</sub> the diffusion constant for oxygen. At a constant rate of oxygen uptake, the oxygen pressure at a locus (phase boundary or structure behaving as an electrode) within the cell will have a constant or flux equilibrium value, P<sub>c</sub>. The electrical polarity of a cell will be determined by at least two oppositely oriented loci and may be expressed as

$$E_p = -RT/2F \ln P_{c_1}/P_{c_2}$$

where the subscript 1 designates the locus of high positive potential as measured externally. The negative sign is conventional only. It was assumed that the velocity constant k<sub>1</sub> is smaller at the locus of high positive potential and that this is the principal difference in the reactions at the two loci. For a tissue, the polarity potentials of the individual cells will sum algebraically, or  $E = \sum E_p$ .

P<sub>c</sub> may be expressed as a function of the oxygen pressure of the medium, P, and the velocity constants of the respiratory reactions, so that the relation of E to P can be inferred. The equations predict that (1) E will be zero when P is zero; (2) as P increases E will increase to a maximum, decreasing again to zero as P becomes indefinitely large; (3) the increase and decrease will be reversible. The position of the maximum on the P axis can only be determined empirically.

<sup>1</sup> Aided by a grant from the Rockefeller Foundation for work on cellular physiology.

In the present paper experimental verification of the second and third predictions is presented for the E.M.F. of the onion root tip. The first prediction has already been essentially realized (5).

### Apparatus and method

The pressure chamber was a cylinder of 2800-cc. capacity made of one-half inch steel pipe. A cover was held in place by twelve bolts through its circumference and through a flange welded to the top of the cylinder. A milled ring on the cover sealed against an asbestos gasket on the flange. A wooden frame held the chamber steady when the bolts were tightened. The cover was provided with an escape valve, a pressure gage reading to 1000 lb. per square inch and a safety valve set to release at 1000 lb. The cover could be tightened to retain 900 lb. for several hours, although as a matter of convenience it was usually permitted to leak slowly. Above 900 lb. there was leakage through the safety valve. The source of pressure was a standard oxygen cylinder connected to the chamber by copper tubing. Water-pumped nitrogen (stated to be 99.8 per cent. pure) was obtained in a cylinder fitting an oxygen needle valve.

The onion bulb was held in a spring clamp to the support of a rack and pinion stand and was wrapped in a sheet of rubber dam to provide insulation and to protect against injury. The root tip was immersed to about 1 mm. in the fluid of the apical electrode cup. The latter was a 65-mm. glass funnel whose shape minimized any rise in fluid which might accompany the solution of large quantities of gas. The basal electrode cup made contact with some region of the root 10 to 20 mm. from the tip, by means of a glass side-tube ending in a loose-fitting claw. With proper adjustment of the initial height of fluid, a considerable volume change could occur in the cup without affecting the contact. A Petri dish of distilled water was placed in the chamber to provide a moist atmosphere.

The electrodes were Ag:AgCl, sealed with DeKhotinsky cement into small glass tubes, and were frequently recoated with chloride. They gave erratic and drifting potentials in tap water, but were stable in one part Ringer's solution to nine parts tap water, which was used as the fluid in the electrode cups.

The measuring instrument was a Compton quadrant electrometer with one quadrant grounded. The basal electrode lead was soldered to the interior of the chamber and the whole grounded from the outside. The apical electrode made connection with the other quadrant through a copper wire baked into the core of a 14-mm. spark plug<sup>2</sup> screwed into the chamber wall, so that the potential measured was that of the tip with respect to the base.

<sup>2</sup> Two such plugs were provided through the courtesy of the Champion Spark Plug Company of Toledo, Ohio, to whom grateful acknowledgment is made.

The pressure gage was scaled in 20-lb. steps and was read to the nearest 5 lb. The E.M.F. was recorded at 30-second intervals during or immediately following any change in pressure, and at longer intervals as it approached a steady value. The pressure was usually elevated abruptly; decompression was carried out more slowly, to permit exchange of gases with a minimum of disturbance to the root or to the electrode fluids. The roots were examined for injury following each experiment by tests of turgidity, opacity, and subsequent growth. There was no evidence that pressures up to 1000 lb. had any injurious effect.

### Results

The effect of oxygen, and the existence of a maximum in the E.M.F. curve, is shown in figure 1. E is the inherent E.M.F., P the oxygen pressure, curve,

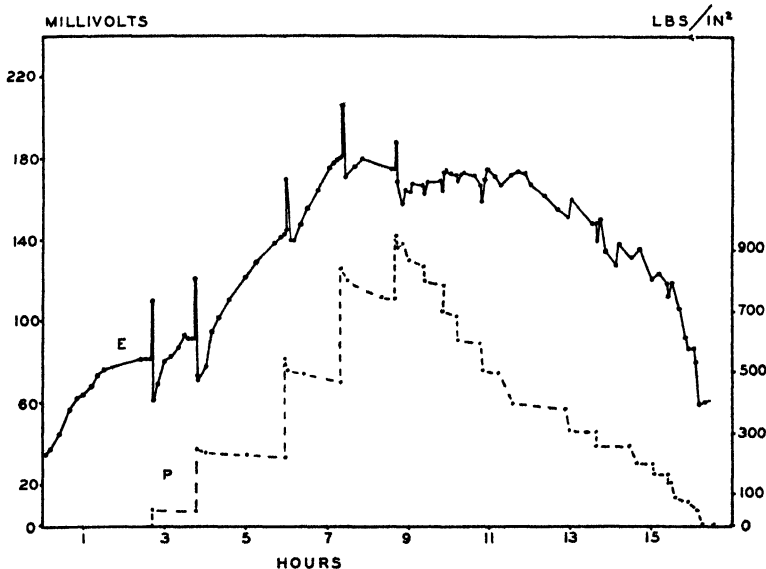


FIG. 1. Potential: time curve for the root at different pressures of oxygen. E, root potential, scale to left; P, oxygen pressure, scale to right. Root tip submerged to 1 mm., basal electrode cup 13 mm. from tip.

plotted against time. In the initial portion of the curve, the root was exposed to air in the chamber. After the E.M.F. had reached a steady value of 82 millivolts, oxygen was admitted to a pressure of 40 lb. The E.M.F. rose instantly to 110 mv., dropped to 62 mv., then gradually climbed to a new steady level of 92 mv. A similar cycle of changes in E.M.F. followed the changes in pressure from 40 to 230 lb. and from 230 to 500 lb. When the pressure was increased to about 800 lb., the final level of E.M.F. was 5 mv. less than that at the lower pressure. Increase of pressure to around 900 lb.

was followed by a further decrease. The pressure was then released in smaller steps. Each abrupt decompression was followed by a cycle of change of E.M.F. roughly the reverse of that produced by an increase of pressure. As the pressure fell from 900 to 600 lb. the E.M.F. rose, to decline again as the pressure was still further reduced. At the end of the experiment, the E.M.F. was about 60 mv. The maximum with increasing pressure lay between 500 and 800 lb.; with decreasing pressure it lay between 800 and 600 lb.

The response of another root to oxygen pressure is shown in figure 2.

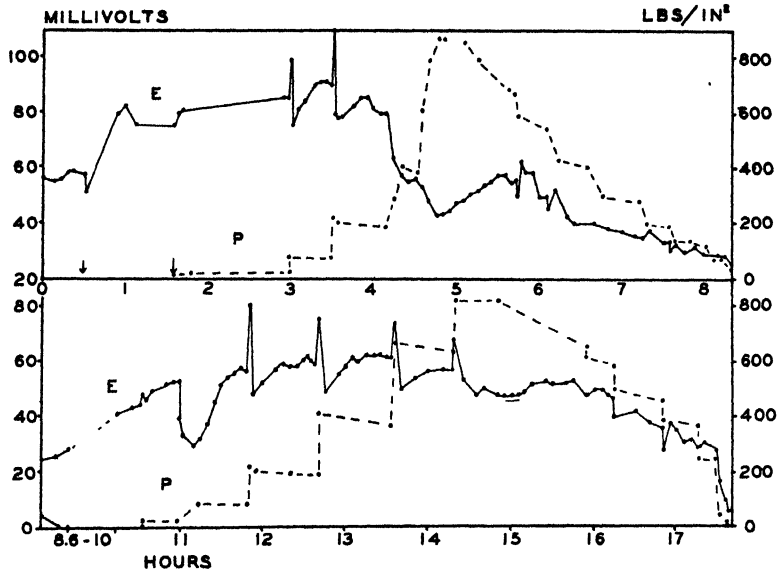


FIG. 2. As in figure 1. Basal electrode cup 26 mm. from tip.

For one hour and five minutes (between the arrows) oxygen was passed through the chamber with the escape valve open. The valve was then closed and the pressure elevated. The E.M.F. increased with the first two pressure increments; but when the pressure was raised from 75 to 200 lb. it dropped 10 mv. and continued to fall as the pressure was increased to 400 and to 860 lb. With release of pressure there was a small recovery with a maximum of about 600 lb., then a steady decline until the pressure had nearly reached zero. For two hours the root was left undisturbed in an atmosphere of oxygen, during which time the E.M.F. rose to a steady level some 10 mv. below that at the beginning of the experiment. The pressure was again increased, the maximum E.M.F. now appearing between 350 and 650 lb. Once more, as the pressure was released, the E.M.F. underwent a small but definite recovery, passing through a maximum as the pressure fell below 700 lb. The last three pressures at which the maximum appeared are in rough agreement.

Ten roots showed the phenomenon of a maximum E.M.F. with increasing oxygen pressure. Two other roots gave increasing potentials up to 570 and 520 lb. respectively, the highest pressures employed. Three roots gave erratic results, showing no consistent effect of oxygen pressure. In general the position of the maximum is well marked with increasing pressure, less well so with decreasing pressure. In part this is apparently due to a slower adjustment of the E.M.F. to a steady level with decreasing pressure, as may be seen in the first decompression in figure 2. It was also generally true that the maximum E.M.F. reached on decompression was less than that reached with increasing pressure.

The electrodes alone were repeatedly tested in the chamber with a salt bridge connecting the electrode cups. Their potential did not vary with oxygen (or nitrogen) pressure.

In order to determine whether the above effects were due to oxygen, as such, or to changes in the physical environment associated with alteration of pressure, the potential of four roots were followed with nitrogen at different pressures. Calculation shows that temperature effects from changing pressure-volume or vapor-pressure relations in the chamber are negligible. The pressure, however, might act as a mechanical stimulus; or temporary evaporation of water from, or deposition upon the root surface could conceivably be responsible for all or part of the changes in E.M.F.

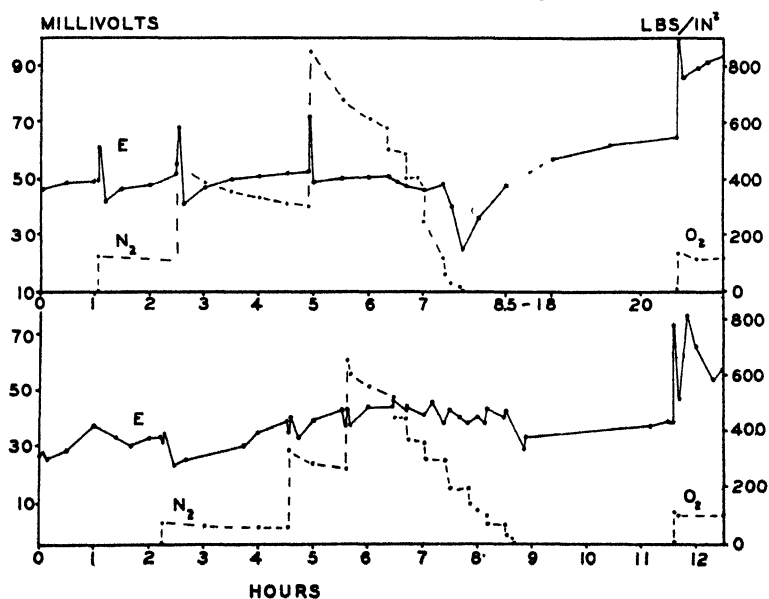


FIG. 3. Effect of nitrogen upon potential of two roots. N<sub>2</sub>, nitrogen pressure; O<sub>2</sub>, oxygen pressure. In both curves, root-tip submerged to 1 mm., basal cup 10 mm. from tip.



Figure 3 shows two such experiments. At the start the chamber was filled with air. In the upper curve each elevation of nitrogen pressure produced a sharp rise and fall of E.M.F., following which the initial value was approximately reached. No change in E.M.F. occurred on decompression until the last 100 lb. was released, when the potential fell to about half its steady value and recovered. The root remained in the chamber over night, as indicated by the break in the curve. Oxygen was then admitted to 110 lb. pressure, producing a large increase in E.M.F. which was maintained above the level in nitrogen for some two hours beyond the end of the curve.

The lower curve of figure 3 is essentially similar. The small increase in potential which occurred during exposure to nitrogen is probably to be attributed to the normal variation in potential (2) since the same rate of increase may be seen when the root was exposed to air. At the end of the experiment, oxygen was passed into the chamber to 100 lb. and the E.M.F. rose from 38 mv. to become steady around 52 mv.

It is evident that pressure alone is not responsible for the changes in the steady level of E.M.F., and that the oxygen pressure relation is a real one. The transition curves from one steady level to another, following an abrupt change in pressure, appear with nitrogen, although their magnitude and character are different from the corresponding curves with oxygen. The difference is particularly striking when obtained upon the same root, as in figure 3.

### Discussion

The experiments presented in this paper are concerned more with the demonstration of the existence of a maximum in the E.M.F.-pressure curve than with the precise determination of the pressure at which the maximum appeared. The most serious limitation to that precision lies in the extreme lengths of time involved in completing a single experiment. As may be seen in figures 1 and 2, one to two hours or more are required for the E.M.F. to attain a new steady level following a pressure change. The maximum is apparently not a pressure point, but a pressure range. This is to be expected upon the principle of summation of potentials (2, 4), for there is no reason to expect the maximum to be the same for all cells of the root.

The position of the maximum varies from one root to another, and there is evidence of a relation between the pressure at which it appears, and the normal potential of the root in air. Further experiments upon this point are now in progress.

The existence of the maximum is a specific prediction of the equations for the flux equilibrium for respiration and E.M.F., and is a consequence of no other mechanism for the production of potentials in living cells. It may, therefore, be regarded as a proof of the validity of the flux equilibrium concept.

### Summary

1. As the oxygen pressure of the environment of the onion root tip is increased, its inherent E.M.F. increases, passes through a maximum, and then decreases.

2. The effect is reversible; *i.e.*, the maximum is found with both increasing and decreasing oxygen pressure.

3. Neither pressure, as such, nor altered vapor tension is responsible for the effect, since the E.M.F. is constant in different pressures of nitrogen.

4. The existence of the maximum fulfills a specific prediction of the equations for the kinetics of an oxidation-reduction system for respiration and E.M.F. at flux equilibrium.

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# VALUE OF THE DYE-ADSORPTION TEST FOR PREDETERMINING THE DEGREE OF HARDINESS

STUART DUNN

## Introduction

The problem of the determination of cold hardiness of plants by methods other than that of the natural one of survival under field conditions has been the subject of numerous investigations during recent years. The chief purpose of most of this work was to evolve a laboratory test that could be used instead of more uncertain and longer field tests.

The dye-adsorption test, in which hydrophilic colloid content is measured colorimetrically by the amount of a dye, such as malachite green adsorbed from aqueous solution, has been shown to offer some promise in the measurement of such material and has given a strong indication that colloidal constituents are in considerable degree responsible for hardiness (2). It was also found that there were great variations in hardiness of individual plants, such as cabbage, in a group chilled simultaneously and that dye adsorption values also varied, but that when a plant first was found to be hardy or non-hardy by a freezing test and then some of its tissue subjected to a dye-adsorption test, the amount of dye adsorbed was found to be correlated with hardiness.

In view of these individual variations and because no hardiness test can be regarded as of practical value if it is not sensitive enough to determine hardiness in advance of cold treatment, it seemed desirable to determine the sensitivity of the dye-adsorption test in that respect.

## Experimentation

In the experiments to be reported *Bryophyllum* and cabbage plants were used. *Bryophyllum* plants are easy to grow and they produce a large number of leaves; which factor is important in sampling. At the same time they allow a sufficient number of leaves to remain on the plant to demonstrate injury by freezing. Cabbage plants have much the same qualities and are also rather hardy.

### EXPERIMENTS WITH *BRYOPHYLLUM*

For the first series of determinations a large number of large-sized, vigorous plants were chosen and divided into several groups varying in number from 10 to 16 plants. A composite sample of several leaves from each plant in a given group was taken and macerated and tested for dye adsorption by the usual colorimetric procedure (2). One-gram samples were treated with 25 cc. of 0.02 per cent. malachite-green solution. As soon thereafter as pos-

sible all the plants in that group were subjected to a temperature of  $-1.1^{\circ}\text{C}$ . for 15 hours. Previous work had shown this to be the right testing temperature. The degree of injury was noted by expressing the results as percentage of the whole plant uninjured or surviving this temperature treatment and this theoretically relates to the amount of dye adsorbed by the tissue. Seven groups of plants comprising a total of 86 individuals were treated in this way and the results, as summarized in table I, show a wide variation in

TABLE I

COMPARISON OF DYE ADSORPTION VALUES WITH FREEZING RESULTS FOR WHOLE  
*BRYOPHYLLUM* PLANTS

SURVIVAL	DYE ADSORBED	MEAN	RANGE
%	%		
100	90-88-84-60-60-46-37	66	90-37
95	57-	57	
90	75-53	64	75-53
85	70-35	52	70-35
80	58-51	54	58-51
75	55-28-28	37	55-28
50	75-70-59-54-50-44-31	54	75-31
40	51-	51	
35	45-35	40	45-35
30	57-	57	
25	58-55-45-18	44	58-18
15	70-59-42-35	51	70-35
10	63-57-54-51-42-31-29-28-17	41	63-17
5	72-68-60-55-54-51-39-15	51	72-15
0	78-75-71-70-67-66-66-65-64- 64-62-57-56-55-55-54-53-53- 53-51-50-49-48-48-45-43-38- 36-34-33-29-21	53	78-21

survival, with several plants totally uninjured, a much larger number entirely killed, and a scattering number intermediately injured. This table has been arranged to show the dye adsorption results for each individual plant grouped together on the basis of their percentage of survival and showing the range and mean value of the dye adsorption results for the plants in each survival group. Inspection of these results shows that for the survival groups containing several plants the range between the high- and low-adsorption values is very large, showing some correlation with hardiness for some plants and none at all in others. The mean values for the different survival groups also show no definite trend of agreement with survival.

Sampling from so large a unit as a whole plant gave results so inconclusive, that a series of experiments was undertaken to secure more representative sampling from smaller, more localized portions of the plant. To this end a group of large *Bryophyllum* leaves was selected and detached

from the plants. They were each halved lengthwise on one side of the midrib and the half with the midrib was placed with the petiole-end in a flat of moist sand, to be tested for its resistance to injury by freezing. Again this plant is rather well adapted to this form of test. Because of its heavily cutinized epidermis it does not wilt readily under these conditions. The other half-leaf was macerated and tested for dye adsorption in the usual way, care being taken to record the halves by number for comparison of results. Five different groups of leaves, making a total of 64, were treated in this way, the leaves in any one group being in one flat and chilled simultaneously. The effect of the exposure to cold again was expressed in percentage of the half-leaf not injured, and the results on all of the different lots are summarized in table II. The results show a large number of leaves entirely

TABLE II  
COMPARISON OF DYE ADSORPTION VALUES WITH FREEZING RESULTS FOR SEPARATE  
*BRYOPHYLLUM* LEAVES

SURVIVAL	DYE ADSORBED	MEAN	RANGE
%	%		
100	66-59-52-48-39-38-35-30-21 11-5	36	66-5
95	95-74-59-44-29-25-5	47	95-5
90	56-54-52-43-41-31-26-14-10	36	56-10
85	60-10	35	60-10
75	50-	50	
70	53-	53	
50	44-	44	
40	15-	15	
35	34-	34	
25	40-36-16	30	40-16
15	37-34-	35	37-34
10	55-20	37	55-20
5	44-25	34	44-25
0	70-61-59-59-56-46-41-40-40 39-34-34-31-28-26-24-13-5 5-4-4	34	70-4

uninjured or slightly injured, a large number totally killed, and a few scattering ones with intermediate injury between. Also the large variation in the range of dye-adsorption values for the large groups of survival percentages is again encountered, as in the results for whole plants, and the mean values show no greater agreement to hardiness. The conclusion to be drawn from the experiments with *Bryophyllum* is that the dye-adsorption test is not effective in predicting hardiness in advance of freezing under the conditions of these experiments.

#### EXPERIMENTS WITH CABBAGE

This type of experiment was extended to cabbage, but, because the cabbage leaf will wilt more readily if detached from the plant for any length

of time, the half-leaves were left attached to the plant. The plants were quite large and after sampling, the whole plant, together with others in the group, were exposed to a temperature of  $-5^{\circ}\text{C}$ . for 15 hours. Two different groups of plants were tested, making a total of 40 plants, and the results appear in table III. No greater agreement of the dye-adsorption results

TABLE III

COMPARISON OF DYE ADSORPTION VALUES WITH FREEZING RESULTS FOR SINGLE CABBAGE LEAVES

SURVIVAL	DYE ADSORBED	MEAN	RANGE
%	%		
85	57-	57	63-47
75	63-47	55	
60	54-	54	
50	50-	50	53-52
35	55-52-	53	
30	51-50-49-48	49	
25	47-	47	57-34
20	57-54-46-40-34	46	
15	67-56	61	
10	61-52-43-42-38	47	61-38
5	45-	45	
0	69-58-56-56-55-55-52- 50-44-42-41-41-40-39	49	

with hardiness is evident here than with *Bryophyllum*.

Since these results indicate that the dye-adsorption test is not accurate enough to predict hardiness of small units of a plant such as a leaf, a further series of experiments was carried out to determine if it can predict

TABLE IV

COMPARISON OF DYE ADSORPTION VALUES ON COMPOSITE SAMPLES WITH FREEZING RESULTS FOR GROUPS OF CABBAGE PLANTS

GROUP NO. AND CONDITION OF PLANTS	LOT	NO. OF PLANTS PER LOT	TREATMENT	TISSUE SURVIV ING	DYE AD- SORBED
				%	%
1 Good-sized, vig- orous plants	A	10	Hardened 7 days at $+5^{\circ}\text{C}$ .	73	40
	B	10	Hardened 5 days at $+1^{\circ}\text{C}$	70	44
	C	10	Unhardened	38	35
2 Old plants	A	12	Hardened 4 days at $+5^{\circ}\text{C}$	71	56
	B	12	Unhardened	77	63
3 Very old plants	A	8	Hardened 6 days at $+5^{\circ}\text{C}$	25	64
	B	8	Unhardened	20	63
4 Young plants	A	25	Hardened 6 days at $+5^{\circ}\text{C}$ .	85	67
	B	25	Unhardened	65	63

with any accuracy the average performance of a large group of plants. Several different groups of cabbage plants of various ages were tested at different times. In each case half of the group was hardened by exposure to a low temperature slightly above  $0^{\circ}$  C. nightly for a few days, in an attempt to secure differences in cold reaction for comparison. Before being exposed to the lethal temperature of  $-5^{\circ}$  C. for 15 hours each group was sampled for a dye-adsorption test by taking one fairly representative leaf from each plant. These were then macerated together and composite samples taken. A summary of the results appears in table IV. Here the results are not as conclusive as could be desired, especially in the second and third groups of old plants where the differences for the values of both tests are slight, but at least the hardier group of plants always gives the greater adsorption. It should be noted that hardening does not always produce a hardier lot of plants, as is shown in group 2.

### Discussion

The literature on various tests and measurements of plant hardiness is very extensive and has been reviewed at great length in several recent papers. Therefore, none of it will be touched on here save to agree with MARTIN (3) and MAXIMOV (4): that none of the methods so far developed can be used with certainty to determine hardiness in a practical way as a substitute for a natural or artificial freezing test. Furthermore, it should be pointed out that the accuracy of many of these methods is determined by comparison with the varietal standings on the basis of general field and observational experience which, from results reported here and in previous papers (1, 2), is frequently very unreliable.

### Summary

1. The dye-adsorption test involving colorimetric measurement of colloid content of plant tissues had previously shown some promise as a measure of cold hardiness. Further work is here reported to determine its accuracy in predicting hardiness in advance of freezing.

2. Tests on individual plants and even on individual leaves of *Bryophyllum* and cabbage gave contradictory results in comparison to subsequent freezing effects on the same plants and leaves, and indicate that the reliability of the test in prediction of hardiness is doubtful. This is similar to the conclusion reached for about every other method for testing hardiness reported in the literature.

3. A somewhat limited effectiveness of the method is indicated for tests of average hardiness of a large group of plants.



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## ENVIRONMENTAL CONDITIONS INFLUENCING THE DEVELOPMENT OF TOMATO POCKETS OR PUFFS<sup>1</sup>

ARTHUR C. FOSTER AND EVERETT C. TATMAN

The condition known as tomato "pockets" or "puffs" is a serious disease or abnormal condition of the fruit of this crop in the Atlantic and Gulf Coast States, and frequently in California. This abnormal condition of the fruit is more prevalent in the mid-winter and early spring crops grown in Florida, and especially in the early spring crops grown in Mississippi and Texas. Frequently 15 per cent. of the total crop is lost in Texas, and often individual growers will lose as much as 65 per cent. of their crop. The malady is also of frequent occurrence on tomato crops grown in greenhouses in the north.

The literature relating directly to tomato "pockets" is limited and almost entirely of an empirical nature. However, there is an abundance of literature which relates indirectly to the phenomenon that contains considerable carefully collected experimental data. As early as 1892, MUNSON (3) observed that adequate pollen distribution was necessary for ovule fertilization and normal fruit development. WHITE (10) observed that insufficient pollen induced seedless tomato fruit with empty locules, which resulted in fruits of the typical pockets type. RADSPINNER (4) and SMITH (7), working in Oklahoma on the relation of environmental factors to anthesis and blossom-drop of tomatoes, came to the conclusion that hot dry winds and low soil moisture favor unusual elongation of styles which resulted in sterility and blossom-drop. SMITH and COCHRAN (8) reported that temperature had a marked effect on germination of pollen and rate of pollen tube growth. HOWLETT (2) reported that carbohydrate deficiency resulted in the suppression of the male organs and pollen sterility, while nitrogen deficiency did not affect microsporogenesis and the development of the male gametophyte.

The literature relating more directly to tomato pockets is rather fragmentary. SANDO (5) tried to determine the influence of various fertilizer combinations on the development of tomato pockets without definite results. He also failed to find very marked chemical differences between pocketed and normal fruit. PRITCHARD<sup>2</sup> working in Florida from 1924 to 1928, made extensive varietal trials with tomatoes and found that Marvelosa produced less pocketing of fruit than other varieties grown. SKINNER and RUPRECHT (6), working in Florida, reported that unfertilized tomatoes developed 80 per cent. of pocketed fruit, and that fertilized plants developed only 30 per

<sup>1</sup> See abstract under same title in *Science*, n.s. 86: 21-22. 1937.

<sup>2</sup> Preliminary report of experimental work on tomato disease. Bureau of Plant Industry, Reports of Progress, 1924 to 1928 inclusive. Unpublished data.

cent. of pocketed fruit. TRAUB, HOTCHKISS, and JOHNSON (9), working in Texas, gave the first detailed description of the malady and established a tentative classification of the different types. They also suggested the use of the term "pockets" in preference to the colloquial term "puffs." FRIEND (1), working in Texas, carried out extensive variety and fertilizer trials with tomatoes and came to the general conclusion that pocketing was not materially affected by the use of fertilizer, but that less loss occurred on plots receiving 600 pounds of superphosphate per acre. He also noted that, while all varieties tested were susceptible to pocketing, some appeared to be more susceptible than others. YARNELL (11), working in Texas, reported that pocketing was often the result of genetic factors affecting fruit development.

The present experiments were conducted in the greenhouse at Arlington Experiment Farm near Rosslyn, Va., during 1931 to 1935. The methods of procedure were rather unique. The plants were grown in a good quality of greenhouse soil made up of clay loam from Arlington Farm, composted with manure and muck, to which sand was later added. Before use, all soil was passed through a  $\frac{1}{4}$ -inch mesh wire screen to insure uniformity. This soil had a water-holding capacity of 65 per cent. of its dry weight, and the water-holding capacity remained uniform over a period of 5 years' experimental work.

The plants were grown in a wide range of approximate soil moisture levels, varying from 39 to 85 per cent. of the water-holding capacity of the soil. The soil moisture series were set up by first determining the original amount of water in the soil, and then adding sufficient water to bring the water content up to the required percentage. The soil moisture was maintained at approximately the desired level by frequent (4 to 6 times daily) additions of measured amounts of water, the required amounts being determined by weighing the cultures on solution balances of 40-kgm. capacity. All plants were grown in 3-gallon glazed crocks holding 12 kgm. of soil.

Fertilizer containing varying amounts of nitrate of soda, superphosphate, and potassium sulphate, at the rate of 2 tons per acre, was incorporated into the soil as it was placed in the crocks. During the 5-year experimental period, fertilizers with widely varying analyses, in the order of N, P, and K, respectively, were used: 0-12-12, 2-2-12, 2-12-2, 8-8-8, 12-0-12, 12-2-2, 12-12-0, and check or no added fertilizer, were used during 1931 and 1932; and two series of crops with these analyses were grown for two short and two long day periods. During 1933, other analyses were used: 0-0-12, 0-6-6, 0-12-0, 6-0-6, 6-6-0, 12-0-0, and check. Another crop was grown during midsummer of 1933 with fertilizer analyses varied and lime added, to obtain varying pH values of the soil from 4.5 to 8.5. In this experimental set-up, the source of nitrogen was varied so as to increase the alkalinity with increasing amounts of nitrate of soda with added lime; and

sulphate of ammonia was used to increase the acidity of the soil. The analyses used in this experiment were: check, 2-6-8, 4-6-6, 6-6-4, 8-6-2, and 10-4-2.

Either 5 or 6 crocks of each fertilizer treatment were placed in each of the 3, 4, or 5 soil moisture series, and these were grown under as uniform temperature as could be maintained in a greenhouse. A crop was matured during December or January for the short daylight period effect, and a duplicate was grown to mature during the middle of summer for the long daylight period effect.

During 1933 to 1935, the fertilizer analyses used were reduced to three: 12-0-12, 12-6-12, 6-12-6, and check or no added fertilizer.

In these experiments, 5 crocks of each fertilizer treatment were placed in each of the three soil moisture series, and these were replicated in each of the three different temperature units of the greenhouse, maintained at approximately 65°, 70°, and 75° F. This gave for study, 5 replications of each at the 36 points of observation, a total of 180 plants under approximately controlled environmental conditions.

The Marglobe variety of tomato was used throughout the experimental period of 5 years. The date of sowing the seed for the short and long day crops varied, but the seedling plants were transplanted to the 3-gallon crocks when about 3 weeks old, and allowed to become established. The plants were staked with heavy wire stakes and later, with the appearance of the first flower cluster, the wire stakes were replaced with 6-foot bamboo stakes. The plants were pruned to a single stem, tied to the stake, and eventually topped after the appearance of the sixth flower cluster. During the growth period, the moisture content of the soil was kept approximately constant by the method already mentioned. A careful record was kept of the total amount of water consumed by each plant daily for the duration of the various experiments.

The experimental method as outlined would indicate that the plants were grown under approximately uniform controlled environment, and this was true for the majority of the cultures. However, to determine the influence or effect of sudden changes in environment, selected plants from the different soil moisture and temperature cultures were changed to other environments and effects observed.

During the 5 years' intensive study of the environmental factors, namely, soil moisture, relative proportion of mineral nutrients, temperature, and length of day period, which influence the development of tomato pockets, a large amount of data has accumulated. These data represent a study of each individual plant, as to its daily and total transpiration rate and water requirement, and also a study of the effects of sudden increases or decreases in soil moisture. The effects of sudden changes in temperature and in evap-

oration capacity of the air have also been studied. The detailed data are too extensive to present in tabular form in a résumé, so a summary of the major results are given.

The most important conditions within the plant and the associated environmental factors that appeared to contribute to the pocketing of tomato fruits in these studies may be classified in three groups, and are as follows:

(1) *No fertilization of ovules or typical parthenocarpy.* It should be recognized that the culture of tomatoes in the greenhouse under varying environmental conditions offers ideal conditions for producing pocketing of the fruit. Under ordinary circumstances pollen distribution is poor but this was largely overcome by frequent agitation of the plants, by handling them in weighing to correct for water loss, in tagging the blossoms as they opened, in the tying of the plants to the stakes, and in the taking of notes. In addition, the most prominent factors that contribute to sterility and parthenocarpy may be briefly noted: (a) abnormally long styles, a result of high temperatures; (b) slow germination of pollen, a result of low temperature; (c) slow growth rate of pollen tube, due to low temperature; (d) pollen abortion, a result of low carbohydrate reserve, caused by high nitrogen, high soil moisture, high temperature, and short daylight period; (e) ovule or embryo abortion, due to low nitrogen reserve, a result of low nitrogen, high soil moisture, high temperature and short daylight period. In these studies the many factors enumerated contributed strikingly to the development of pocketed fruit, especially in the high and low temperature greenhouse units when large amounts of nitrogen were applied.

(2) *Ovule or embryo abortion after normal fertilization.* The saturation or supersaturation of the soil appeared to cause marked changes in the normal metabolic, respiratory, and transpiration activities of the plant, which, in turn, resulted in ovule or embryo abortion. Also, excessive drought accompanied by high transpiration, a condition that apparently results in endoxerosis, causes marked apparent changes in normal metabolic, respiratory, and transpiration activities, and results in ovule and embryo abortion and the development of pockets.

(3) *Necrosis of vascular and placental tissue after fruit growth is well developed.* During any period of the growth of the plant, the saturation or supersaturation of the soil apparently stops almost entirely all transpiration, causes marked changes in color, the plants becoming chlorotic, and also otherwise interferes with the normal metabolic activities. These conditions apparently contribute largely to the appearance of necrosis of the vascular and placental tissue, which, in turn, leads to the development of pockets of the fruit of any age. Low soil moisture, accompanied by high transpiration, results in endoxerosis of the vascular and placental tissue. Sudden changes from optimum or high soil moisture to low soil moisture, accompanied by

excessive transpiration, appeared to be the most drastic treatment of all that favored the development of pockets.

Samples for chemical analyses, collected from the stem, bottom part of the plant, middle part of the plant, top part of the plant, and of fruit of different ages show marked differences in carbohydrate and nitrogen content. Significant correlations are to be expected with reference to the different plant parts and the appearance of pockets. The analyses of fruits from various treatments differ little when collected at the same stage of maturity, with the exception of those grown in the low temperature house. They are almost entirely vegetative in nature, and have a higher percentage of total solids, reducing sugars, and starch.

In these studies we are dealing with an environmental complex of many factors, as, for instance, soil moisture, soil nutrition, air temperature, light duration, and the interrelation and interaction of these component factors, any one of which may become a limiting factor to normal plant growth, and metabolic and respiratory activity, and thereby interfere with the normal development of tomato fruits. There is considerable suggested evidence that large amounts of superphosphate and only moderate amounts of nitrogen in the fertilizer reduce pockets by giving a nutritional balance conducive to more nearly normal seed development. The factors that can be observed and measured appear, when adverse, to bring about a general disturbance of the metabolism of the plant, causing a condition of suboxidation or endoxerosis, to affect the  $\text{CO}_2$  and  $\text{O}_2$  exchange, which, in turn, apparently leads to a visible necrosis of the vascular and placental tissue, thereby affecting ovule and embryo and placental development and normal fruit growth.

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## PHOTOPERIODIC STIMULATION OF GROWTH BY ARTIFICIAL LIGHT AS A CAUSE OF WINTER KILLING

PAUL J. KRAMER

(WITH ONE FIGURE)

The results of several recent investigations indicate that length of day or photoperiod is an important factor in determining the length of growing season in certain species of woody plants. The short days of late summer and autumn stop stem elongation and the production of new leaves in a number of species long before temperatures are low enough to check growth. This has been strikingly demonstrated by a hedge of *Abelia grandiflora* growing on the Duke University campus. *Abelia* is ordinarily quite hardy, but it has been observed that the plants in this hedge near the electric lights winter-kill badly, while those midway between the lights are never injured. A study was therefore made to determine the cause of this difference.

The entire hedge was trimmed September 25 and at that time it was quite uniform in appearance. By October 20, when the first light frosts occurred, the plants on each side of the lights for a distance of about five yards were distinctly different in appearance from those farther away. They bore numerous new shoots which were soft and succulent, and covered with pale, green leaves which contrasted strongly with the short, woody shoots, and bronzed, dark-green color of the leaves farther from the light. Difference in color and amount of growth was so marked that it could be seen at a distance of over 200 yards.

The number of new shoots produced since pruning was counted in a strip one foot wide at yard intervals from the lights. The results of 14 such counts were averaged and are shown in figure 1. It will be observed that, beginning five yards from the light, the number of new shoots decreased rapidly with increasing distance up to about 15 yards, where the number became constant. It was also observed that the plants near the lights continued to flower later in the season than the plants at a distance.

Late in November, after several hard freezes had occurred, counts were made of the number of frosted shoots at yard intervals from the lights. It was found that the tips of all the growing, succulent shoots had been killed back two to six inches. The averages of ten such counts are shown in figure 1. It will be observed that the greatest number of dead shoots occurred at three yards from the lights, which is also the point of greatest light intensity, and that no dead shoots were found at a distance of nine or more yards from the lights.

The light intensity amounted to only about two foot-candles at the brightest point, so it could not be measured satisfactorily with an ordinary



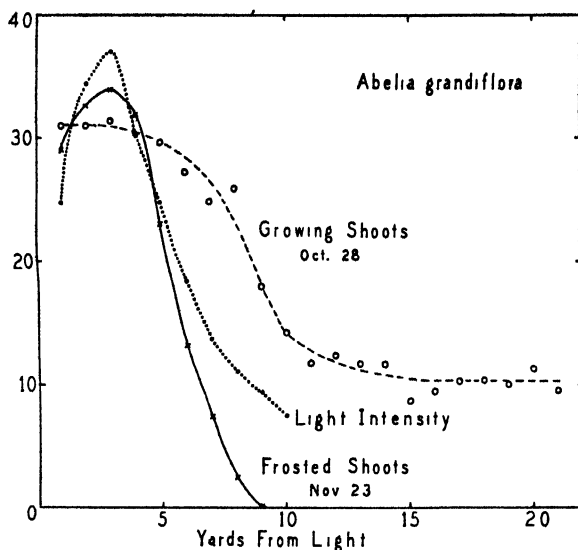


FIG. 1. Number of new shoots developed following last pruning; number of frosted shoots and light intensity measured at yard intervals from lights.

illumination meter calibrated in foot-candles. It was therefore measured at yard intervals from the lights by means of two photronic cells connected in parallel to a microammeter. The curve for light intensity represents the average of five determinations and is plotted in microamperes  $\times 10$ . It will be noted that the number of new shoots produced after the last pruning, and the number of frosted shoots, are closely correlated with light intensity up to about ten yards from the lights. The most marked effects occur in the first five yards, nearly twice as many shoots being killed at five yards as at six yards. Since the maximum intensity was only two foot-candles, a light intensity of less than one foot-candle will produce a photoperiodic reaction in *Abelia*. The entire hedge is exposed to weak illumination from the lights, which burn all night; but at a distance greater than ten yards from the light the intensity is too low to affect growth.

Early in September, two dozen vigorous *Abelia* plants were removed from the nursery, potted, and placed outside the greenhouse. Half of them were exposed to normal length of day while the other half received daylight supplemented with sufficient electric light to give a photoperiod of about 14.5 hours, which equals the longest days of midsummer. By early October, only two of the normal-day plants had produced new shoots, while all of the long-day plants bore long, succulent shoots with light-green leaves. The new growth was killed back by November freezes, while the woody shoots and bronzed, dark-green leaves of the normal-day plants were entirely uninjured. It appears that a photoperiod equal to that of midsummer is just as effective

in prolonging the growing season as the continuous light to which the hedge is exposed.

Black locust (*Robinia pseudoacacia* L.) seedlings exposed to long and normal photoperiods reacted in the same manner as *Abelia*. The normal-day plants ceased growth in September while the long-day plants continued rapid growth until killed by freezing weather in November. The normal-day plants were entirely uninjured by these freezes.

### Discussion

The results of these experiments clearly indicate that winter killing resulted from the failure of the *Abelia* to cease growth and become hardened before freezing weather occurred. The failure to cease growth can definitely be attributed to the photoperiodic stimulation of electric light. It is evidently unwise to plant *Abelia* too near bright lights. The same conclusions apply to black locust.

These results are in accord with those of certain Russian workers who have stressed the importance of photoperiodic reaction in determining the degree of cold resistance of woody plants. BOGDANOV (1) and MOSHKOV (3, 4) found that certain southern species transferred to the long days of northern Russia continued to grow in the autumn until killed by frost. If the photoperiod was artificially shortened they ceased growth before freezing weather occurred, and survived the winter without injury. As the writer (2) has reported, not all trees are sensitive to a long photoperiod, and it is unlikely that all shrubs are sensitive. It is believed, however, that many instances of winter killing of isolated trees and shrubs result from proximity to electric lights causing growth to continue too late in the autumn. Several such occurrences have already been reported to the writer, and it is hoped that other investigators will search for similar examples. Information of this nature will enable those in charge of planting operations to avoid disappointments by keeping light sensitive plants away from bright lights.

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## BRIEF PAPERS

### DEVELOPMENT OF CAROTENOID PIGMENTS WITHOUT THE AID OF LIGHT

Xanthophyll and carotene are usually associated in plants and are present before the formation of chlorophyll. Their formulae suggest genetic relationship. Xanthophyll can be reduced to carotene and the transformation is reversible. Together they may represent a respiratory mechanism similar to the haemoglobin-oxyhaemoglobin complex in animal forms.

No chlorophyll develops in seedlings not exposed to light as was first recorded by JOANNES RAJUS in 1693. BONNET called such plants "etiollées" and the condition is now known as etiolation.

ARNAUD in 1889 first suggested that etiolated leaves contain carotene. The carotenoids are in leucoplastids which are generally assumed to be identical with the chloroplastids that contain the chlorophyll of normal plants. KRAUS first studied the alcoholic extract of etiolated leaves spectroscopically and concluded that the yellow pigments and the chlorophyll of normal plants are genetically related. PRINGSHEIM made similar studies in 1874 and concluded that a special pigment was present which he called "etiolin." This substance seems to be identical with protochlorophyll.

The results of experiments by the writer and by others before him, prove that chlorophyll forms within less than 30 seconds after etiolated plants are exposed to light. Protochlorophyll is probably the precursor.

According to observations of the writer the yellow color of etiolated sunflower seedlings is limited almost entirely to the cotyledons. The stems are white except for a small zone directly below the cotyledons. Upon exposure to light, this zone increases downward, the yellow extending beyond the green when chlorophyll forms. In time, however, the yellow and green colors become confused. It appears that some material is translocated in the stem which is converted into pigment in the plastid by the metabolic processes of the protoplasm.

The writer's experiments further show that the carotenoids are formed in sunflower seedlings without the aid of light. Some seeds contain carotenoid pigment, but attempts to extract them from sunflower seeds failed. Even if it is assumed that an initial amount was present, careful studies by the writer proved that the pigment forms in the absence of light. The greatest rate of development was between the fourth and seventh days of sprouting. The rate of increment in that time was much greater for carotene than for xanthophyll, and the decline from the seventh to the fourteenth day was much greater for the carotene. The amount of carotene developed, in comparison with the amount of xanthophyll, was always small.

The xanthophyll:carotene ratio increased during the period from the fourth to the seventh day, from 9 to 12, and during the period from the seventh day to the fourteenth day, from 13 to 20.

It may be concluded that the origin and the function of carotene and xanthophyll are probably related. The material stored in the seed probably is the source of the necessary material and energy required for the formation of the carotenoid pigments.

Carotene appears to have a stimulating effect on the production of roots, which suggests that carotene may be a precursor of auxin.—WILLIAM A. BECK, *Institutum Divi Thomae, Graduate School of Scientific Research, Cincinnati, Ohio.*

## NOTES

**Annual Election.**—The annual election of the American Society of Plant Physiologists has resulted in the choice of the following officers for 1937–1938: President, Dr. OTIS F. CURTIS, Cornell University; vice president, Dr. WALTER F. LOEHWING, The State University of Iowa; secretary-treasurer, Dr. F. P. CULLINAN, United States Department of Agriculture, Beltsville, Maryland; elected member of the executive committee, Dr. R. B. HARVEY, University of Minnesota (term to expire in 1940); elected member of the editorial committee, Dr. H. R. KRAYBILL, Purdue University (term to expire in 1940).

Two vacancies have been filled by executive committee action. Dr. BURTON E. LIVINGSTON, The Johns Hopkins University, has been appointed to the executive committee for one year, vice the election of the retiring president to a three year term by ballot. To succeed Professor FRANCIS E. LLOYD, McGill University, who after twelve years of faithful service has resigned from the editorial committee, Dr. G. W. SCARTH, McGill University has been appointed for a five-year term.

**Denver Meeting.**—The meeting of plant physiologists in Denver was arranged under the joint auspices of the American Society of Plant Physiologists, the Western Section of the society, and the Physiological Section of the Botanical Society of America. The meeting opened on June 22 with a symposium on chlorosis and some of the minor elements. The Western Society for Soil Science joined in the meeting, and an attendance of over 100 attested the wide interest in this subject. The afternoon session of June 22 was devoted to a symposium on plant hormones under the leadership of Dr. F. WENT, California Institute of Technology. This symposium was exceptionally well arranged, and presented the developmental history of hormone research, supported by current investigations. The discussion was continued during the evening at a dinner for plant hormone physiologists at the Cosmopolitan Hotel. The dinner was attended by more than 40 interested scientists. Round table discussion was punctuated or accompanied by brief sketches of work in progress, and by a free discussion of controversial points by men having different concepts and points of view. The deep interest shown in this program indicates that scientists really enjoy the stimulus of such meetings, and should probably provide for skilfully developed round table discussions more frequently.

The morning of June 23 was occupied with the presentation of numerous short papers, and the afternoon was spent with section G, A.A.A.S., which was celebrating its 100th meeting. June 24 was featured by a symposium on drought resistance in plants, with Dr. A. H. HENDRICKSON as chair-

man. A dinner and smoker for plant physiologists on Thursday evening was attended by about 80 individuals. The toastmaster, Dr. J. P. BENNETT, University of California, announced the results of the annual election, and also the election of two new patrons of the society, Dr. BURTON E. LIVINGSTON, The Johns Hopkins University, and Dr. ALEXANDER P. ANDERSON, Tower View Laboratory, Red Wing, Minnesota. He then called upon president R. B. HARVEY, who responded with an address on the processing of citrus fruits, and upon Dr. GEORGE R. HILL, JR., who responded with an address on the control of smelter gas injury to vegetation.

Friday morning was again given over to a program of numerous short reports, and the afternoon to field trips; however, heavy rain dampened the enthusiasm for mountain climbing and picnicking.

The meeting as a whole reflects great credit upon the officers of the several organizations cooperating in its arrangement. It was as well attended, as successful, and as full of interest as the usual meetings in December.

**New England Section.**—The annual meeting of the New England Section was held at the Rhode Island State College, Kingston, Friday and Saturday, May 14–15, 1937. An interesting program of short papers was arranged for Friday afternoon. This covered such problems as auto-irrigation of cultures, pH and soluble iron from ferric phosphate, embryo development of ash seeds previous to germination, influence of Bordeaux spray on tomatoes, calibration of recording illuminometers, sampling of light under forest canopies, and the use of cellulose films for supplementary insulation of plants under glass.

Friday evening was given over to the annual dinner, with an address of welcome from President BRESSLER, and an illustrated lecture on controversial conservation by HENRY E. CHILDS.

Saturday morning was planned to give opportunity to observe the field plots, greenhouses, and laboratories of the Rhode Island Agricultural Experiment Station, and the State College. Another session for presentation of papers followed the tour of the experimental fields where so many interesting discoveries have been made. In the midst of this program the annual business meeting was held as an intermission period. The officers of the New England section elected for 1937–1938 are Dr. F. H. STEINMETZ, University of Maine, chairman; Dr. H. B. VICKERY, Connecticut Agricultural Experiment Station, vice chairman; and Dr. L. H. JONES, Massachusetts Agricultural Experiment Station, was reelected secretary-treasurer. The fourth annual meeting of the section will be held at Orono, Maine, in May, 1938, under the auspices of the University of Maine.

Saturday afternoon was devoted to a teachers' forum which attracted much attention. It was a seminar on the teaching of plant physiology, deal-

ing with the logical objectives and methods of approach in teaching plant physiology. The committee members in charge of this new departure were Dr. C. J. LYON, Dartmouth College, chairman, Dr. DOROTHY DAY, Smith College, Dr. CARL G. DEUBER, Yale University, and Dr. F. H. STEINMETZ, University of Maine. This meeting was so significant and so valuable that a special report from the chairman of the forum committee will be published in the October number of *PLANT PHYSIOLOGY*. It is a sign of healthy progress that deep interest is being shown in the different pedagogical techniques demanded in handling plant physiology in the agricultural colleges, and in liberal arts colleges. It is hoped that future seminars along similar lines may bring the problems of effective teaching of this subject to the forefront of interest among plant physiologists throughout the United States.

**Corporate Seal.**—The American Society of Plant Physiologists was incorporated as a non-profit organization under the laws of the District of Columbia, July 5, 1932. Under the revenue laws relating to exemption of non-profit organizations from income taxes, it was necessary to have a corporate seal. During the last year the secretary has given attention to the development of an appropriate design, which was submitted to the executive committee at the Denver meeting for approval. The executive committee authorized the adoption of a corporate seal, approved with slight change the design submitted, and authorized the secretary to have it made up immediately. We hope that a reproduction of the design may be printed as soon as it is available.

**Legislative Committee.**—The executive committee authorized the appointment of a legislative committee at the Denver meeting, the vice president of the society to be chairman of the committee. Dr. C. O. APPLEMAN, University of Maryland, was named for a two-year term on the committee, and Dr. CHARLES B. LIPMAN, University of California, to a three-year term. Dr. W. F. LOEHWING, vice president, will be chairman during 1937–1938.

**Hans Molisch.**—It has seemed appropriate to honor those who have been elected as corresponding members of the American Society of Plant Physiologists by some form of public recognition of their service. It is therefore with great pleasure that we are able to present in this number of *PLANT PHYSIOLOGY* a portrait of HANS MOLISCH, accompanied by a paper from L. LINSBAUER on *Fifty years of Plant Physiology in Austria*.

HANS MOLISCH was born at Brünn (Mähren) on July 12, 1856, and celebrated his eightieth birthday anniversary last year. His parents were JOHANN and FRANZISKA (MATZA) MOLISCH. He was educated at the K. K. Deutschen Ober-Gymnasium at Brünn, and at the University of Vienna,



which institution he entered in 1885. On March 10, 1888, he was married to IDA KNOLLER, who is the mother of two children, Paul, born in 1889, and Fritz, born in 1894.

In 1889 HANS MOLISCH became ausserordentlicher Professor at the Technische Hochschule in Graz. Several years later he moved to Prag, where in 1894, he was appointed ordentlicher öffentlicher Professor in the German university and director of the Plant Physiological Institute. In 1909 he returned to Vienna with the same title. During 1922 to 1925 he was visiting professor at the Imperial University at Sendai, Japan, and travelled extensively in the Orient. Since 1925 he has been at the University of Vienna as emeritus professor of plant physiology. Throughout these years of his professional life he was very active in his investigations, and has published over 200 papers on plant physiology. He is also well known for his many books (about 20), some of which were standard textbooks used in the universities, while others served to increase popular interest in physiological experiments. He was elected a corresponding member of the American Society of Plant Physiologists at St. Louis in 1935.

At the beginning of his eighty-second year, we are happy to extend to him the cordial greetings of American plant physiologists, who appreciate the great contributions he has made to our knowledge of plants during the last half-century, and who admire him as an indefatigable worker in this field of science.

**Edward C. McCarty.**—News of the sudden death of Dr. EDWARD C. McCARTY at his home in Riverside, California, on December 31, 1936, at the age of 52 years, came as a severe shock to his many friends throughout the country. Dr. McCARTY was born in 1884, and held degrees from the Jefferson School of Law (Doctor of Laws, 1912), from the University of Montana (B.S., 1916), and from the University of California (Ph.D., 1927). He was associate professor of plant physiology at the Colorado Agricultural College from 1920 to 1926, and during the last 10 years had been on the faculty of the Riverside Junior College. Here he combined in happy manner his aptitude as a teacher, his fondness for investigation, and his love of students. His research on range problems attracted attention of the U. S. Department of Agriculture and he was employed during the summer in the work of the Forestry Division. His work on grasses and their responses to grazing are particularly noteworthy. One of these contributions was published in *PLANT PHYSIOLOGY* 10: 727–738. 1935.

Gifted with an unusually friendly personality, he was a wise counsellor and friend to all of his students and colleagues. His industry, his honesty, his loyalty to his associates and to his own ideals, his humility of spirit endeared him to all who knew him; and his departure represents an irreparable

loss to his family, his friends, his institution, and to his field of science. He was for several years a member of the American Society of Plant Physiologists.

The immediate cause of death was a heart attack. Funeral services were held on the morning of January 4, 1937, at the First Congregational Church in Riverside.

**Annual Review of Biochemistry.**—The sixth volume of the *Annual Review of Biochemistry* has just been published. It is not only a very creditable example of the art of book making; it is also a volume whose usefulness and value would be difficult to overstate. The reviews are carefully prepared. Everyone who has tried to review the progress of any given field must realize the difficulty of the task undertaken in this series; and it is remarkable that year after year the work is so ably done. This indicates a faithfulness and responsibility on the part of editors and reviewers that is worthy of great praise. No biochemist, no physiologist can afford to omit it from his list of indispensable reference works.

The 1937 volume contains 28 reviews, many of which are of interest to plant physiologists. Some of the most important of these are mentioned to give some idea of the scope of the review. The first paper is on permeability, by R. COLLANDER; others are on biological oxidations and reductions, by F. LIPMANN; enzymes, by K. LINDERSTRØM-LANG; the application of microchemistry to biochemical analysis, by P. L. KIRK; the chemistry of carbohydrates and glycosides, by W. N. HAWORTH and E. L. HIRST; the chemistry of the lipins, by E. KLENK and K. SCHWIRTH; the chemistry of the steroids, by R. SCHOENHEIMER and E. A. EVANS, JR.; the chemistry of the proteins and amino acids, by C. S. ADAIR; detoxication mechanisms, by A. J. QUICK; plant pigments, by J. H. C. SMITH; the alkaloids, by E. SPÄTH; photosynthesis, by R. EMERSON; mineral nutrition of plants, by F. G. GREGORY; organic acids of plants, by T. A. BENNETT-CLARK; and biochemistry of bacteria, by C. B. VAN NIEL.

The other reviews on metabolism, vitamins, hormones, etc., are mainly reviews of progress in the animal field. Thus a fair balance is maintained in the entire field of biochemistry. It represents an outstanding value among recent scientific works. An important valuable addition to volume VI is a subject index. This feature is so useful, and so necessary to rapid reference to the material that it should be included in each succeeding volume.

The editors, reviewers, and publishers deserve the success which has attended this series from its inception. Volume VI costs but \$5.00, and may be ordered from Annual Review of Biochemistry, Ltd., Stanford University, California.

**The Minor Elements.**—A second edition of the *Bibliography of References to the Literature of the Minor Elements and their Relation to the Science of Plant Nutrition* has been issued by the Chilean Nitrate Educational Bureau Inc., 120 Broadway, New York. The original edition of 1935 was compiled by Prof. L. G. WILLIS, soil chemist at the North Carolina Agricultural Experiment Station. It contained about two thousand abstracts, and was so popular that this first edition was soon exhausted. The new edition contains 396 pages, and nearly a thousand new references and abstracts. It is a very valuable compilation and ought to be in the hands of every plant physiologist. Those who find references that are not included in the second edition are invited to send them to the Chilean Nitrate Educational Bureau. Those who wish to obtain a copy of the second edition should write to Mr. HERBERT C. BREWER, director of the Bureau. As the supply is already low, there is a possibility that some may have to be disappointed. They are supplied free of charge as long as they last.

**Monograph on Growth.**—Under the title *Studies on Wheat Grown under Constant Conditions*, the Food Research Institute, Stanford University, California, has published a valuable monograph on the growth of the wheat plant. The author of the monograph, Dr. H. L. VAN DE SANDE-BAKHUYZEN, will be remembered for his papers published in PLANT PHYSIOLOGY in 1928. The material of those papers is incorporated in the monograph, with a large mass of new data. He was assisted in parts of the work by ELIZABETH P. GRIFFING, and Dr. CARL L. ALSBERG of the Food Research Institute. There are seven sections or parts to the monograph, with 36 chapters in all. The main divisions of the monograph bear titles as follows: Part I, the growth curve in annual plants; II, methods and materials; III, general growth features; IV, dry weight and moisture content of the various organs; V, dry weight and moisture of the standard plant; VI, nitrogen and carbon of the organs; and VII, nitrogen metabolism in relation to growth and development. In addition to numerous figures and tables, there is a bibliography with 233 citations, and a brief index. The entire work occupies 400 pages.

Few agricultural plants have been investigated as carefully as the wheat plant has been for the preparation of this monograph. The program of research was both extensive, and intensive. A very good beginning has been made toward the elucidation of the growth behavior of wheat. This monograph should stimulate studies of other important plants along similar lines. Plant physiologists and students of general growth problems will find it of great value. The price of the monograph is \$4.00 per copy, and orders may be addressed to the Food Research Institute, Stanford University, California.

**The Nature and Properties of Soils.**—This well-known textbook by Dr. T. L. LYON and H. O. BUCKMAN of Cornell University has appeared in a third edition. The work has been thoroughly revised to keep it in line with the changing emphasis in the study of soils. Some changes have been made also in the organization of the material, so that there are now only 17 chapters, some of which are new. One of these, chapter IV, deals with colloidal clay and ionic exchange. All of the material is valuable to the plant physiologist, and the last 5 chapters present a valuable survey of the problems of the maintenance of soil fertility. These are: Liming the soil; the nitrogen economy of soils; fertilizers and fertilizer practice; farm-manure and green-manure; and the methods of fertility maintenance for mineral soils. The book has not been much increased in size, it maintains its readable style, is not too technical for average students, and should make friends of all who are engaged in the problems of soil science and plant science. The book may be ordered from Macmillan Co., at \$3.50 per copy.



VLADIMIR NIKOLAEVITCH LUBIMENKO  
JANUARY 18, 1873—SEPTEMBER 14, 1937

# PLANT PHYSIOLOGY

OCTOBER, 1937

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## PLANT PHYSIOLOGY IN THE U.S.S.R.

V. N. LUBIMENKO

The extraordinary richness and variety of plant life on an enormous territory, embracing almost one-sixth of the land surface of the world, naturally impelled the Russian botanists first to direct their efforts to the study of the specific composition of its flora and of the geographic distribution of the plants. Classification and phytogeography were therefore the first branches of botany to be developed and still remain the predominant botanical disciplines in the U.S.S.R., both in the number of scientific specialists and in the amount of scientific research work accomplished.

Nevertheless, the beginning of plant physiology refers to the sixties of the last century, the founder of the first physiological school being A. S. FAMINTSYN (1835–1918), Professor of the St. Petersburg University and member of the St. Petersburg Academy of Sciences (since 1889). He published the first university text-book on plant physiology (1887) and a large monograph *Metabolism and transformation of energy in plants* (1883). His first pupils were BARANETZKY and BORODIN.

Plant physiology began to develop towards the end of the 19th and in the beginning of the 20th century, and there appeared consecutively the schools of C. A. TIMIRIASEV, V. I. PALLADIN, S. P. KOSTYCHEV, D. N. PRIANISHNIKOV, A. A. RICHTER, V. V. K. ZALESSKY, V. N. LUBIMENKO, N. A. MAXIMOV, N. G. KHOLODNY.

There was, however, an especially marked rise in the development of plant physiology after the October Revolution, which gave a great impetus to the organisation of new universities, high schools, botanical gardens, and large agricultural experimental institutions, wherein laboratories of plant physiology began to appear. Cadres of young physiologists of the new generation began to grow rapidly. In the Soviet laboratories they receive a complete special education. A great change is also to be observed in the general tendencies of research work. The old direction in plant physiology, as reflected in large handbooks and in text-books, in essence gave only a physico-chemical analysis of separate, unconnected functions of the organ-

ism; the organism as a living, dynamic, whole system was completely absent from them.

At the present time the attention of experimental workers is being more and more centered on the organism itself and on its proper internal factors, controlling all the physico-chemical reactions occurring within it. Consequently not only *phylogenetic variability* (manifesting itself in different species, races, and varieties, in connection with ecological adaptation to environment) is now taken into account even in ordinary biochemical investigations, but likewise *individual variability*, characterizing different stages of ontogenetic development.

The influence of each separate external factor on one or another reaction in the organism is not now studied by means of its simple isolation, as was the method formerly, but on the background of different combinations of other factors. As a result there has been produced an essential modification in our notions of the cardinal points, minimum, optimum, and maximum in physiological functions. They have ceased to be immovable points of a single curve; they have become dynamic, as a reflection of the dynamism of the organism itself, of the change in state of the protoplasm. The earlier investigations on photosynthesis (LUBIMENKO, KOSTYCHEV, L. A. IVANOV, and their collaborators, CHESNOKOV and BAZYRINA) have now given along this new line of investigation and study most interesting results in the works of A. C. DANILOV (the influence of light of different spectral composition), V. A. BRILLIANT (the influence of the water content of leaf tissue), A. K. TOSHCHÉVIKOVA and A. L. KURSANOV (the influence of the accumulation of assimilants in the leaf).

The same line of research is followed in the studies on frost resistance, heat resistance, drought resistance (N. A. MAXIMOV, I. I. TUMANOV, A. A. RICHTER, V. N. LUBIMENKO, and collaborators), on mineral nutrition (O. A. WALTER, D. A. SABININ and their collaborators), and on nitrogen nutrition (D. N. PRIANISHNIKOV and collaborators).

The problems of growth and growth movements have shown, after the old works of PORODKO, NELJUBOV, and ROTHERT, an exceptionally brilliant development in the works of N. G. KHILODNY, who has together with WENT created the hormone theory of growth.

During the last ten years a special and most important place has been won by works on the physiology of development of the higher plants. GARNER and ALLARD's discovery of photoperiodism, and LYSENKO's discovery of the temperature yarovisation of seeds have served as a powerful impulse in directing research work along this path.

The favorable results in agricultural practice of the method elaborated by LYSENKO of the preliminary yarovisation of seeds have led to a wide development, in the greater part of the physiological laboratories of our

Union, of research work on the physiology of growth and development of plants. Attempts are now already being made of synthesizing all of the accumulated data into a theory of development (the most important theory of development-stages of **LYSENKO**, the hormone theory of **LUBIMENKO**, **KHOLODNY**, **CHAILAKHIAN**). Data have also been obtained for the practical application of photoperiodism in plant culture (the investigations of the laboratories of **LUBIMENKO** and **MALCHEVSKI**). An essential feature of the physiological research work of the present day is the choice of the problems studied. They all concern questions having the greatest practical significance for the agriculture and industry of the U.S.S.R. The necessity of such a choice of problems is the natural result of the necessity of replacing the empirics which formerly prevailed in industrial practice by methods scientifically elaborated and approved.

It is of course self evident that such an organization of work does not in the least exclude investigations of the most profound theoretical questions, which are studied in the laboratories of our academies and universities.

At the same time the socialistic structure of the whole economy of the U.S.S.R. guarantees a very speedy penetration into practice of valuable scientific attainments and discoveries. The following may be cited as an example: **LYSENKO** began to apply his method of preliminary yarovisation of seeds to field cultures in 1931, and already millions of hectares are being sown with yarovized seeds.

In conclusion I wish to emphasise that the high estimation of scientific research work, both by the Government and by society, create in the U.S.S.R. most favorable economic and moral conditions for scientific work and fully guarantee the development of science in general, and of plant physiology in particular.

BOTANICAL GARDEN  
LENINGRAD, U.S.S.R.





ASSIMILATION OF AMMONIUM AND NITRATE NITROGEN FROM  
SOLUTION CULTURES BY ROOTS OF *PANDANUS VEITCHII*  
HORT., AND DISTRIBUTION OF THE VARIOUS  
NITROGEN FRACTIONS AND SUGARS IN  
THE STELE AND CORTEX<sup>1</sup>

C. P. SIDERIS, B. H. KRAUSS, AND H. Y. YOUNG<sup>2</sup>

(WITH EIGHT FIGURES)

Introduction

In the course of studies on the assimilation of ammonium and nitrate nitrogen by pineapple roots, to be presented in a future publication, it became necessary to follow the conversion of inorganic to organic nitrogen in small lineal regions of the roots and also in the cortex and stele of these regions. As the diameter of pineapple roots is very small and a separation of the tissues of the stele from those of the cortex presents great difficulties, roots of *Pandanus veitchii* were employed. Shoots from plants grown on the campus of the University of Hawaii were placed in water cultures containing respectively ammonium and nitrate salts as sources of nitrogen.

The conversion of inorganic to organic nitrogen was followed in cortex and stele of different regions of the root either by analyzing such tissues or by analysis of the exudate which was collected in flasks under aseptic conditions from the excised end of a certain number of roots.

Literature review

This being the first of a series of papers to follow on the assimilation of ammonium and nitrate nitrogen by different plant tissues, the literature is reviewed rather broadly from the point of view of the entire subject rather than from that of the paper under immediate consideration.

The literature on absorption and assimilation of ammonium salts as sources of nitrogen by different higher green plants has been presented by PARDE (48) and in a more general manner by MOLLIARD (36). However, for a better understanding of the rôle of ammonium nitrogen in absorption, assimilation, and translocation the reader is referred to certain original sources of information. PRIANISCHNIKOW (54, 55, 56, 57) and PRIANISCHNIKOW and IWANOWA (58, 59), who for the last 25 years have studied the various phases of absorption and assimilation of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  at different stages of plant growth, conditions of carbohydrate reserves, pH values, and

<sup>1</sup> Presented at the June, 1935, meeting of the American Society of Plant Physiologists at St. Paul, Minn.

<sup>2</sup> The first of a series. Published with the approval of the Director as Technical Paper no. 95 of the Pineapple Experiment Station, University of Hawaii.

calcium concentrations, found: (1) that  $\text{NH}_4^+$  is absorbed at comparatively greater rates at higher pH values than  $\text{NO}_3^-$  and *vice versa*; (2) that the toxicity characteristic of plants grown in  $\text{NH}_4^+$  containing cultures at low pH values is due to  $\text{H}^+$  ions and not to  $\text{NH}_4^+$ ; and (3) that  $\text{NO}_3^-$  ions are reduced first, in conformity with WARBURG's theory (80), to ammonia and then converted to amino acids or products of the type of  $\text{R}(\text{NH}_2)(\text{CONH}_2)$  (asparagine, glutamine). PRIANISCHNIKOW's findings have been verified and further extended by others with respect to nitrate reduction.

ECKERSON (14) has followed the assimilation of  $\text{NO}_3^-$  ions in different tissues of plants and her findings indicate a reduction of  $\text{NO}_3^-$  to  $\text{NO}_2^-$  and a further one to  $\text{NH}_4^+$ , which results are in harmony with PRIANISCHNIKOW's theory.

The process of  $\text{NO}_3^-$  reduction is not limited to few tissues or organs and may be found to operate in the roots, leaves, and possibly in the stem according to THOMAS (69), NIGHTINGALE and SCHERMERHORN (45), NIGHTINGALE and ROBBINS (44), DAVIDSON and SHIVE (12), TIEDJENS and BLAKE (72), HOLLEY, DULIN, and PICKETT (20), MASKELL and MASON (30), WOO (81), LEONARD (25), and others.

The relative amounts of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  nitrogen absorbed from solution cultures at high and low pH values have been studied by TSUNG-LE LOO (77), EGGLETON (16), LEWIS (26), TIEDJENS (72), TIEDJENS and BLAKE (73), CLARK (8), CLARK and SHIVE (9), DAVIDSON and SHIVE (12), STAHL and SHIVE (64, 65), PIRSCHLE (50, 51, 52), NAFTEL (39), MEVIUS (33), MEVIUS and ENGEL (34), DIKUSSAR (13), NIGHTINGALE (41), PRIANISCHNIKOW (55, 57), etc. Their results indicate that  $\text{NH}_4^+$  ions are absorbed and assimilated at considerably greater rates than  $\text{NO}_3^-$  and that high pH values favor more the absorption of the former and low pH values that of the latter ions.

The effects of different nutrient elements, temperature, light, and other climatic conditions on the absorption and assimilation of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  ions have been studied neither extensively nor very satisfactorily. From the available literature (6, 17, 18, 19, 22, 28, 30, 39, 41, 42, 43, 47, 49, 62, 71, 74, 75) we learn that low temperature, small amounts of light, and exceedingly low concentrations of certain nutrient elements retard absorption and assimilation of nitrogen and other elements, and synthesis of proteins.

### Materials and methods

*Pandanus veitchii* shoots weighing between 1 and 4 kilos and having from 2 to 8 aerial roots were employed for these studies. The roots, already formed on the shoots and varying in length from 10 to 50 cm., and in diameter from 0.5 to 2.5 cm., had not yet come in contact with soil nor had they produced any laterals, and from a functional point of view they were virgin as they had never absorbed mineral substances from an external medium.

The tip and distal end, 10 to 15 cm. of each root, were submerged in tap water contained in individual 2-quart Mason jars until an extensive lateral root system was produced. The roots of all different containers were aerated. The time required for the production of an extensive lateral root system was about eight months. The lateral root systems of the different roots were classified into as uniform groups as possible and they were then subjected to various treatments. Certain of such roots were used exclusively for the study of the chemical composition of their exudates and others for that of their tissues. The roots of both groups were divided into smaller lots for different nutritional treatments. These included two types of complete nutrient solutions containing nitrogen from two different sources, that is, either ammonium or nitrate and a third solution which contained no nitrogen but that was otherwise complete. The chemical composition of these solutions is reported in table I.

TABLE I  
CHEMICAL COMPOSITION OF NUTRIENT SOLUTIONS

SALT	AMMONIUM	NITRATE	MINUS- NITROGEN
	MOL. CONC.	MOL. CONC.	MOL. CONC.
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.0010	.....	.....
(NH <sub>4</sub> ) <sub>2</sub> CO <sub>3</sub> .....	0.0010	.....	.....
KH <sub>2</sub> PO <sub>4</sub> .....	0.0005	0.0005	0.0005
K <sub>2</sub> SO <sub>4</sub> .....	0.0010	0.0010	0.0010
MgSO <sub>4</sub> .....	0.0010	0.0010	0.0010
CaCl <sub>2</sub> .....	0.0020	.....	0.0020
Ca(NO <sub>3</sub> ) <sub>2</sub> .....	.....	0.0020	.....
FeSO <sub>4</sub> * .....	0.0001	0.0001	0.0001
pH (about) .....	6.8	5.6	5.4

\* A stock solution of FeSO<sub>4</sub> · 7H<sub>2</sub>O was prepared by dissolving 346 gm. of the salt in one liter of 0.1/N H<sub>2</sub>SO<sub>4</sub>. It was kept in a dark bottle and used in the dilution indicated above.

The roots of the plants in the exudate experiments were severed from the stem at the time they were placed in the different nutrient solutions. The cut-off end of the root was led, in all cases, into the mouth of a 125-cc. Erlenmeyer flask, containing 1 cc. of toluene, where it discharged the exudate which was removed from the flask at 24-hour intervals. In cases in which roots were allowed to discharge for more than 48 hours, a portion of the cut-off end, about 0.5 to 1 cm. long, was removed to secure an unobstructed flow and prevent contamination of the exudate with the by-products of dying tissues. The roots of the group for studies of the chemical composition of the tissues included small lateral roots and the main root fractions

which were obtained by crosswise cutting into three parts, (1) the terminal, (2) the intermediate, and (3) the proximal fraction of the main roots. With the exception of the small lateral roots, the tissues of the stele were separated from those of the cortex. All the tissues were weighed before they were placed in individual containers and were analyzed separately. Three different samples were prepared for chemical analysis of each group of tissues: (1) for ammonia and organic nitrogen analysis; (2) for nitrate and sugars; and (3) for drying. In the sample for sugar analyses a small volume of  $(\text{Na})_2\text{CO}_3$  was added to prevent hydrolysis.

The exudates were analyzed as rapidly as the volume necessary for analysis was collected. The tissues of the roots harvested for analysis were cut into small pieces and placed for 30 seconds in flasks containing a small volume of boiling water (for inactivation of enzymes). The containers were immediately cooled and 5 cc. of toluene added. All samples were analyzed within a period of 2 to 4 weeks.

#### PREPARATION OF SAMPLES

The plant tissues were next strained through small pieces of cheesecloth. The collected liquid was returned to the flask while the solids were placed in a brass mortar and ground with quartz sand. The ground solids in a fine pulpy state were returned to the original flask containing the liquid. Methods generally employed in biochemical analyses with recent modifications (29) and improvements have been adopted for ascertaining the chemical composition of the exudates and tissues.

#### ANALYSES FOR AMMONIA AND ORGANIC NITROGEN

AMMONIA.—The entire volume of the ground tissues and liquid was placed in pyrex cylinders, 35.5 cm. long and 4.45 cm. in diameter, to which N/1 NaOH was added to bring to pH 8 and then 5 to 10 cc. depending on the quantities of tissues, of a phosphate-borate buffer of pH 8 (61) was added. The battery of 12 cylinders was placed in a temperature bath, maintained at 45° C. The contents of the cylinders were aspirated at 45° C. for 1 hour for small and for 3 hours for larger quantities. The ammonia of the tissues was collected in N/50  $\text{H}_2\text{SO}_4$  and was determined by *Nesslerization* or by titration, depending on the amounts present.

GLUTAMINE.—The ammonia-free residue was brought with  $\text{H}_3\text{PO}_4$  to pH 5 to 6. It was then boiled for 2 hours, evaporated to a smaller volume, cooled, brought with N/1 NaOH to pH 8 and aspirated at 70° C. The ammonia was then determined as already described.

#### SEPARATION OF SOLUBLE FROM INSOLUBLE ORGANIC NITROGEN

The glutamine-free residue was cooled, acidified with acetic acid to pH 3, and toluene was added. It was allowed to stand in the refrigerator over-

night or at room temperature and then filtered. The residue on the filter paper (no. 42 Whatman) was repeatedly washed with boiling water containing 0.5 per cent. acetic acid. The filtrate was very clear and apparently free from suspensions of proteinoid substances. The residue, containing the proteins of the tissues, was placed in a flask together with a definite volume of 20 per cent. HCl and stoppered for later analysis. The filtrate containing different fractions of soluble organic nitrogen was then analyzed according to the following methods.

#### ANALYSIS OF SOLUBLE NITROGEN FRACTIONS

ASPARAGINE.—To the total volume of the filtrate,  $\text{H}_2\text{SO}_4$  was added to make a 4 per cent. concentration. The mixture was heated, under a reflux condenser, for two hours, and sufficient NaOH was added to make it distinctly but not excessively alkaline (pH 8 to 9). The ammonia formed from asparagine was aspirated at  $70^\circ \text{C}$ . as in the method for ammonia. The amounts of amide nitrogen thus obtained were multiplied by 2 to include the amino nitrogen of asparagine. However, the amino nitrogen value was subtracted from the alpha-amino of mono-amino determinations.

ALPHA-AMINO NITROGEN.—About 20 cc. of the asparagine-free residue was adjusted with  $\text{H}_2\text{SO}_4$  to pH 6 and was then evaporated on a water bath to a volume of 4 cc. Two cc. of the residue were employed for the determination of alpha-amino nitrogen, using the method of VAN SLYKE (78). The modified reaction vessel of KOCH (24) was employed and both temperature and barometric pressure were recorded with each determination for calculating the weight of  $\text{N}_2$  gas.

MONO-AMINO NITROGEN.—In certain determinations instead of alpha-amino nitrogen mono-amino nitrogen is reported. This fraction was recovered in the filtrate from the residue of the asparagine determination after treatment with phosphotungstic acid. It is essentially composed in its major portion of mono-amino-carboxylic acids and mono-amino-dicarboxylic acids, and it was obtained as follows: The asparagine-free residue was acidified with 30 cc. of concentrated  $\text{H}_2\text{SO}_4$ , diluted to 300 cc. and then treated with 10 cc. of 50 per cent. phosphotungstic acid depending on the volume of the residue and amounts of basic nitrogen contained therein. The mixture kept at about  $4^\circ \text{C}$ . for 40 hours was filtered through no. 42 Whatman filter paper applied to a Buchner funnel, which was fitted through a hole made in a can of greater diameter than the funnel and containing ice. The precipitate was washed repeatedly with acidified phosphotungstic acid at about  $5^\circ \text{C}$ .

The filtrate was placed in a Kjeldahl flask where it was digested by adding 5 gm. of anhydrous sodium sulphate and 5 drops of selenium oxychloride. After cooling and the addition of 40 per cent. NaOH, the generated ammonia was distilled in 0.05 N  $\text{H}_2\text{SO}_4$ .

**Basic nitrogen.**—This fraction representing the quantities of nitrogen contained in such amino acids as arginine, histidine, lysine, and cystine is included in the phosphotungstic precipitate obtained during the separation of mono-amino nitrogen. The precipitate contains, possibly, small amounts of certain other nitrogenous fractions. The precipitate and filter paper were placed in a Kjeldahl flask where they were digested with a mixture of 25 cc. of concentrated sulphuric acid and otherwise treated as already indicated.

**REST NITROGEN.**—In cases where the extract containing the soluble organic nitrogen was not hydrolyzed beyond the stage of the asparagine determination the residue was employed directly for estimating alpha-amino and rest nitrogen. The alpha-amino was determined by the VAN SLYKE method as already stated. For the estimation of rest nitrogen the previously described Kjeldahl technique was followed on an aliquot of the residue. From the total value of the nitrogen thus obtained that of alpha-amino minus one-half of the asparagine nitrogen was subtracted. The difference represents rest nitrogen which has been possibly derived from some basic nitrogen and from other sources not well known.

#### ANALYSIS OF INSOLUBLE ORGANIC NITROGEN

The residue or insoluble portion of the tissues, obtained after the removal of glutamine nitrogen, was refluxed, as stated under the heading "separation of soluble from insoluble fractions of organic nitrogen," with 20 per cent. HCl for 24 hours. The hydrolysate was neutralized and filtered. The insoluble residue and filtrate were transferred to separate Kjeldahl flasks, the former containing the humin or melanin nitrogen and the latter different fractions of hydrolyzed protein.

**HUMIN NITROGEN.**—The total nitrogen content of the flask composed of the acid resistant particles of the tissues was determined by the Kjeldahl procedure already described.

**AMIDE NITROGEN.**—The filtrate or hydrolysate obtained after the digestion of the dissolved portions of the tissues with 20 per cent. HCl was made slightly alkaline with NaOH, then aspirated, and the ammonium estimated as in the glutamine determination.

**MONO-AMINO AND BASIC NITROGEN.**—The amide-free residue was neutralized, acidified with 20 cc. of concentrated  $H_2SO_4$ , treated with 10 cc. of 50 per cent. phosphotungstic acid, and kept at 4° C. for 40 hours. The determination of the mono-amino nitrogen and basic nitrogen was then made in the same way as already described.

#### ANALYSES FOR NITRATE NITROGEN AND SUGARS

**NITRATE NITROGEN.**—This fraction was determined on an aliquot of the sample employed for sugar analysis, using the phenol disulphonic method (2).

This method was compared with that of PUCHER, LEAVENWORTH, and VICKERY (60) and that of TRESCHOW and GABRIELSEN (76) and was found very satisfactory for the plant material concerned. The tissues were extracted repeatedly with water until the final weight was 10 times greater than the initial weight of the tissues. An aliquot of the extract was cleared with lead acetate and the filtrate from the precipitation was delead with  $\text{Na}_2\text{HPO}_4$ , and filtered. An aliquot of the filtrate was evaporated over a water bath to dryness, then treated with phenoldisulphonic acid, water, and ammonium hydroxide (2). With samples containing certain pigments interfering with the accuracy of the colorimetric determination a small amount of norite was added. After 2 to 4 hours the mixture was filtered and the residue in the filter was washed repeatedly.

**SUGARS.**—The extracts employed for sugar analysis were cleared as stated above and the method of Bertrand as presented by KERTESZ (23) was used for the determination of reducing and total sugars. Sucrose was hydrolyzed with invertase.

### Experimental results

The experimental results are presented in two sections: (1) the chemical composition of the root exudates, and (2) the chemical composition of the root tissues.

#### CHEMICAL COMPOSITION OF ROOT EXUDATES

The data from many experiments summarized in table II show that the

TABLE II

DISTRIBUTION OF DIFFERENT NITROGENOUS FRACTIONS IN THE EXUDATE FROM ROOTS OF *PANDANUS VEITCHII* GROWN IN SOLUTION CULTURES WITH EITHER AMMONIUM OR NITRATE SALTS OR IN THE TOTAL ABSENCE OF AN EXTERNAL SUPPLY OF NITROGEN

NITROGENOUS FRACTIONS	NUTRIENT SOLUTIONS		
	AMMONIA	NITRATE	MINUS-NITROGEN
Inorganic			
Ammonium .....	0.013	0.002	0.003
Nitrate .....	0.000	0.030	0.000
Soluble organic			
Glutamine .....	0.008	0.003	0.002
Asparagine .....	0.016	0.016	0.006
Mono-amino .....	0.162	0.135	0.063
Basic .....	0.077	0.048	0.038
$\alpha$ -amino .....	0.212	0.171	.....
Total organic .....	0.255	0.194	0.109



exudates of the roots grown with ammonium nutrition contained considerably greater quantities of total nitrogen than those grown in the nitrate or minus-nitrogen cultures. They also show that the roots of the nitrate series contained more total nitrogen than those which lacked an external nitrogen supply. The presence of organic nitrogen in the exudates of the roots grown in the minus-nitrogen solutions is exceedingly interesting because it indicates that translocatory nitrogen, derived by hydrolysis of reserve proteins, may be present in fair quantities in the sap of plants grown in minus-nitrogen cultures. Of the different fractions of organic nitrogen alpha-amino nitrogen is the greatest. As it is included mostly in the fractions indicated as mono-amino and basic nitrogen the difference between the value of the sum of the latter two fractions and that of the former is small indicating that other nitrogenous fractions are present in extremely small quantities. Amide either as glutamine or asparagine occurs in small amounts. Occasional exceptions may be found, as in table III, where this fraction is relatively high.

TABLE III

DISTRIBUTION OF DIFFERENT NITROGENOUS FRACTIONS IN EXUDATE FROM ROOTS OF *PANDANUS VEITCHII* GROWN IN SOLUTION CULTURES WITH EITHER AMMONIUM OR NITRATE NITROGEN OR IN THE TOTAL ABSENCE OF AN EXTERNAL SUPPLY OF NITROGEN

NITROGENOUS FRACTIONS	NUTRIENT SOLUTIONS		
	AMMONIA	NITRATE	MINUS-NITROGEN
Inorganic			
Ammonia	0.005	0.007	0.005
Nitrate	0.000	0.135	0.000
Soluble organic			
Amide	0.200	0.055	0.017
Amino	0.490	0.163	0.045
Total organic	0.690	0.218	0.062

Some of the conditions which favor the formation of great quantities of amide nitrogen will be mentioned in the discussion.

Ammonium occurs in very small amounts in the exudate regardless of the kind of nitrogenous salts employed in the nutrient solution because of the great rate of assimilation of this fraction of nitrogen by the root tissues (9, 64, 65, 72, 73). The presence of ammonium, in traces, in the exudate of roots grown in nitrate-containing or minus-nitrogen cultures is due possibly in large part to hydrolyzed proteins. That nitrate might have been reduced to ammonium in the nitrate cultures is also probable. The quantities of ammonium found in the nitrate-supplied plants, however, are small and of the

same magnitude as those found in the minus-nitrogen cultures, but, keeping in mind the great rate at which ammonium is assimilated, its presence in more than traces would scarcely be anticipated in either case (9, 64, 65, 72, 73).

Nitrate may be found in small or large quantities in the exudate of roots owing respectively to an increased or decreased rate of assimilation of this fraction. In tables II and III and figures 2 and 3 it is shown that there was found from 30 to 135 mg. of nitrate nitrogen per liter of exudate. The variations are possibly due to differences in the physiological conditions of the root cells which may favor the reduction and assimilation of nitrates more at certain times than at others. Similar observations have been made in other plants (15, 70).

A point which deserves further emphasis in these studies is the alpha-amino nitrogen which, in nearly all cases, is between 70 and 90 per cent. of the total soluble organic nitrogen in the exudate. No protein either coagu-



FIG. 1. *Pandanus vertohii* shoot with roots of different lengths and diameters but without rootlets which are only produced when the tip is either placed in a nutrient solution or enters the soil.

lable by heat or filterable could be found in the exudate in more than mere traces.

### CHEMICAL COMPOSITION OF ROOT TISSUES

**DISTRIBUTION OF NITROGENOUS FRACTIONS IN THE TISSUES OF ROOTS GROWN IN AMMONIUM OR NITRATE CULTURES.**—The roots grown in the different cultures were cut, as stated previously, into different parts separating the lateral and terminal rootlets from the main root tissues. Also the tissues of the terminal fraction of the main root were separated from those of the intermediate and proximal region as illustrated in figure 1. The basal tissues of

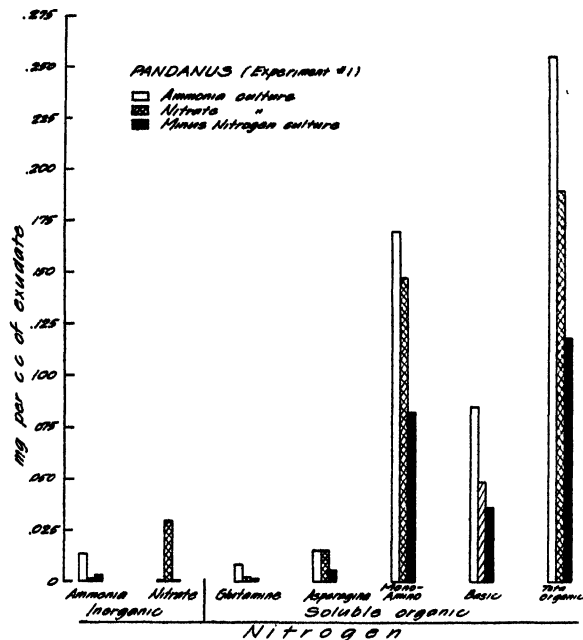


FIG. 2. Distribution of different fractions of inorganic and soluble organic nitrogen in the root exudates of *P. veitchii* grown in ammonium-, nitrate- or minus-nitrogen-solution cultures.

the roots, i.e., those in close proximity to the stem, were not used. A comparative examination of the data in table IV and in figures 4, 5, and 6, with respect to the distribution of the different nitrogenous fractions in the stele and cortex of various regions of the root, discloses the following facts:

**DISTRIBUTION IN THE ROOTLETS AS CONTRASTED IN FIGURE 4.**—1. The amounts of nitrate in the rootlets of the nitrate-supplied plants were considerably higher than the ammonium nitrogen in the rootlets of the ammonium series. These findings are indicative of a lower rate of assimilation of nitrate as compared to ammonium in the rootlet tissues of *Pandanus veitchii*.

2. The glutamine in the rootlets of the ammonium cultures is considerably greater than in those of the nitrate group, indicating a greater rate of glutamine synthesis from ammonium than from nitrate ions.

3. The amounts of asparagine are about the same in the rootlets of both ammonium and nitrate cultures.

4. The quantities of soluble mono-amino nitrogen are greater in the rootlets of the plants grown in ammonium than in those receiving nitrate, while soluble basic nitrogen is of about the same magnitude in both cases.

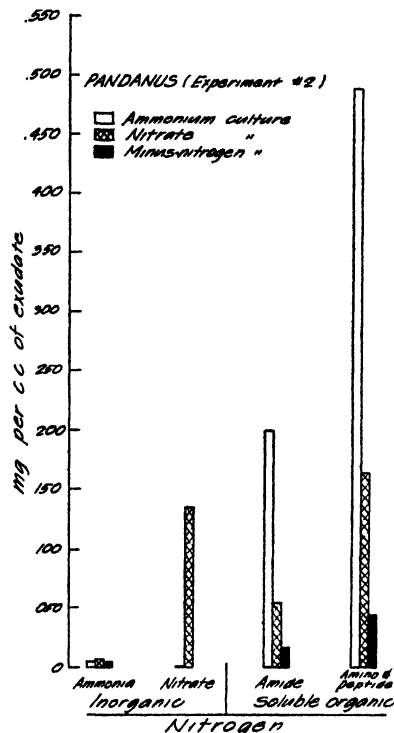


FIG. 3. Distribution of different fractions of inorganic and soluble organic nitrogen in the root exudates of *P. veitchii* grown in ammonium-, nitrate- or minus-nitrogen-solution cultures.

5. The nitrogenous fractions of insoluble nitrogen, with the exception of mono-amino nitrogen, are about the same in the rootlets of the plants of both ammonium and nitrate cultures.

DISTRIBUTION IN THE TERMINAL TISSUES OF THE MAIN ROOT AS CONTRASTED IN FIGURE 5.—1. As in the rootlets nitrate is considerably higher in the terminal tissue of the main root of the nitrate-supplied plants than is ammonium in comparable tissues of ammonium-grown plants. Nitrate is present only in the roots grown in nitrate cultures, whereas ammonium nitrogen

TABLE IV

DISTRIBUTION OF DIFFERENT NITROGENOUS FRACTIONS AS MG. NITROGEN PER GRAM FRESH WEIGHT IN VARIOUS ROOT TISSUES OF *PANDANUS VEITCHII* GROWN IN NUTRIENT SOLUTIONS AND CONTAINING EITHER AMMONIUM OR NITRATE SALTS AS SOURCES OF NITROGEN

NUTRIENT SOLUTION	NITROGENOUS FRACTIONS	MAIN ROOT TISSUES						ROOTLET
		PROXIMAL		INTERMEDIATE		TERMINAL		TISSUES
		CORTEX	STELE	CORTEX	STELE	CORTEX	STELE	
Ammonium-N	Inorganic	mg.	mg.	mg.	mg.	mg.	mg.	mg.
	Ammonium	0.005	0.006	0.004	0.015	0.008	0.019	0.038
	Nitrate	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Soluble organic							
	Glutamine	0.004	0.009	0.008	0.020	0.027	0.045	0.038
	Asparagine	0.090	0.123	0.068	0.133	0.138	0.164	0.127
	Mono-amino	0.436	0.365	0.517	0.667	0.576	0.929	0.770
	Basic	0.231	0.272	0.313	0.540	0.332	0.696	0.345
	Insoluble organic							
	Amide	0.024	0.048	0.057	0.094	0.036	0.116	0.035
	Mono-amino	0.149	0.141	0.113	0.222	0.128	0.228	0.132
	Basic	0.096	0.101	0.120	0.223	0.075	0.324	0.062
	Humin	0.201	0.236	0.201	0.286	0.156	0.353	0.156
Nitrate-N	Inorganic							
	Ammonium	0.004	0.010	.. ..	.. ..	0.008	0.025	0.006
	Nitrate	0.018	0.095	.. ..	.....	0.050	0.100	0.180
	Soluble organic							
	Glutamine	0.008	0.017	.. ..	.....	0.013	0.013	0.002
	Asparagine	0.050	0.182	.. ..	.. ..	0.145	0.352	0.124
	Mono-amino	0.541	0.552	.. ..	.....	0.586	0.863	0.667
	Basic	0.298	0.420	.. ..	.....	0.289	0.555	0.340
	Insoluble organic							
	Amide	0.033	0.135	.....	.....	0.100	0.296	0.031
	Mono-amino	0.050	0.174	.....	.....	0.125	0.385	0.053
	Basic	0.047	0.178	.....	.....	0.090	0.452	0.063
	Humin	0.115	0.239	.....	.....	0.290	0.481	0.151

occurs in the roots grown in cultures containing either ammonium or nitrate. The quantities of ammonium found under both conditions, however, are comparatively small apparently owing to rapid assimilation of ammonium. The quantities of nitrate in the tissues of the stele are greater than in those of the cortex, possibly, because the vessels of upward conduction are mostly located in the former tissues (11, 32).

2. Glutamine<sup>2</sup> is more prevalent in the stele than in the cortex. The quantities found are greater in the roots grown in ammonium than in nitrate

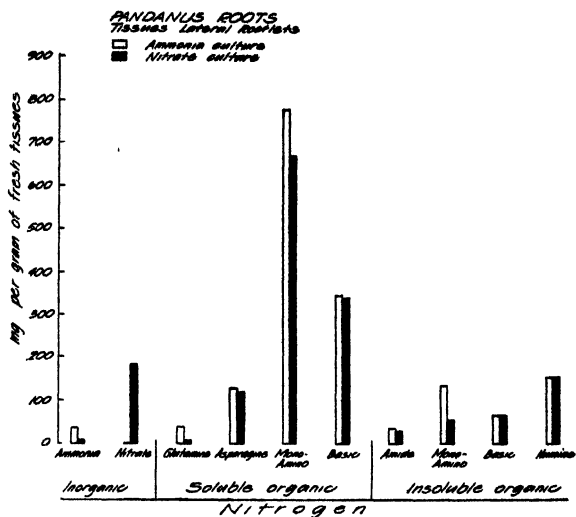


FIG. 4. Distribution of different fractions of inorganic, soluble organic, and insoluble organic nitrogen in the rootlet tissues of plants grown in ammonium- or nitrate-solution cultures.

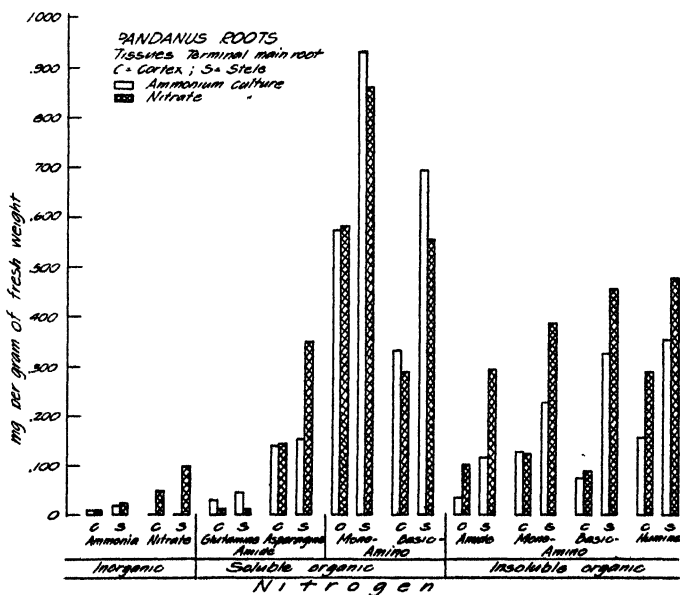


FIG. 5. Distribution of different fractions of inorganic, soluble organic, and insoluble organic nitrogen in the cortex and stele tissues of the terminal section of the main root of *P. veitchii*.

cultures, which condition is associated with a greater rate of ammonium than of nitrate assimilation and also with conditions peculiar to ammonium metabolism which favor the synthesis of great amounts of glutamine as observed by CHIBNALL and WESTALL (7) and VICKERY, PUCHER, and CLARK (79).

3. Asparagine is distinctly more abundant in the stele than in the cortex. Differences between the amounts of asparagine of the roots of ammonium- and nitrate-grown plants are relatively great, indicating a greater rate of synthesis of asparagine from nitrate than from ammonium.

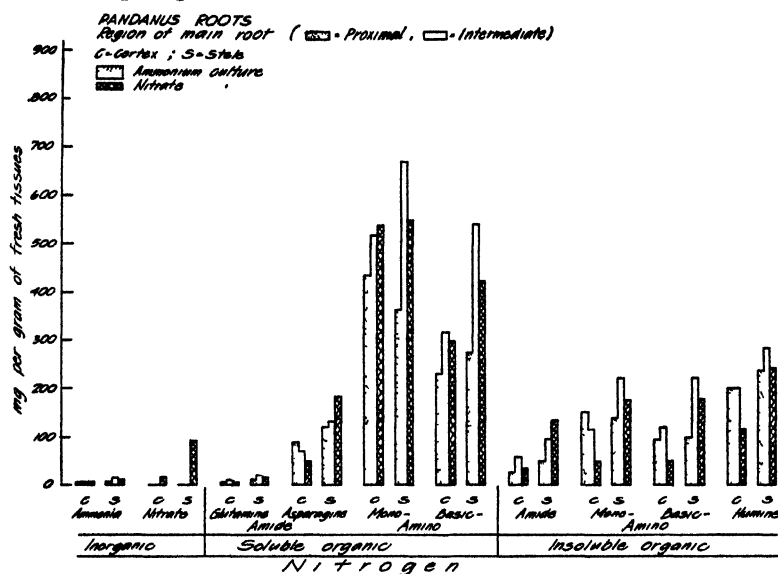


FIG. 6. Distribution of different fractions of inorganic, soluble organic, and insoluble organic nitrogen in the cortex and stele tissues of the intermediate section of the main root of *P. verchou*.

4. The amounts of mono-amino and basic soluble nitrogen are considerably greater in the stele than in the cortex. Also, the amounts of both fractions contained in the roots of plants grown in ammonium are considerably greater than those of plants supplied with nitrate.

5. The various fractions of insoluble nitrogen are higher in the stele than in the cortex. With one minor exception, the amounts of all the fractions of insoluble nitrogen of the roots grown in nitrate are higher than those of the roots furnished with ammonium, indicating that more protein nitrogen accumulates in the roots of nitrate than in those of ammonium-supplied plants.

DISTRIBUTION IN THE INTERMEDIATE REGION OF THE MAIN ROOT AS CONTRASTED IN FIGURE 6.—Analytical values of the intermediate tissues of the

main root are only available for the roots grown in ammonium cultures. These values, contrasted with those of figure 5, show a gradual decrease in the quantities of most nitrogenous fractions possibly owing to greater amounts of cellulosic material, vacuolation, and other such features associated with maturation.

The proximal tissues of the main root of the plants grown in nitrate cultures compare more in age and in general development with the intermediate than with the proximal tissues of the plants receiving ammonium. In comparative observations this point should be taken into consideration.

1. Ammonium in the tissues of roots of plants grown either in ammonium or in nitrate cultures is very low while nitrate in the root tissues of the latter cultures is relatively abundant.

2. Glutamine is very low in the root tissues of both lots of plants.

3. Asparagine is relatively abundant in the roots of plants of both ammonium and nitrate cultures. Certain differences in the content of asparagine in the proximal and intermediate tissues of the cortex cannot be explained satisfactorily.

4. Soluble mono-amino and basic nitrogen values in the tissues of the stele of nitrate-grown plants are lower with respect to the intermediate region of the main root, but higher with respect to the proximal fraction of plants grown in ammonium cultures. Certain variations in the mono-amino nitrogen content of the stele and cortical tissues cannot be explained.

5. The distribution of the different fractions of insoluble nitrogen in the intermediate region of the roots of the two different cultures shows many fluctuations. Comparing the cortex of either the proximal or intermediate portions of the roots grown in ammonium cultures with those of the nitrate series, with the exception of amide, the quantities of all the other fractions of insoluble nitrogen are higher in the roots of the former than in those of the latter series. The amounts of different fractions of insoluble nitrogen in the stele of both lots are greater than in those of the cortex. Also the proximal portion of the roots of the plants grown in ammonium cultures contain, with one exception, slightly smaller amounts of insoluble nitrogen than the intermediate region.

DISTRIBUTION OF SUGARS.—The distribution of sugars in the stele and cortex of different parts of the root, as presented in table V and figure 7, shows that:

1. In the main root total sugars occur in greater amounts in the stele than in the cortex. In the rootlets only very small quantities are found.

2. Reducing sugars occur in both stele and cortex. Their quantities in the stele increase, with minor exceptions, from the proximal towards the terminal portion of the main root, indicating that there is an accumulation of these sugars at the distal end of the main root possibly owing to a de-



TABLE V

DISTRIBUTION OF DIFFERENT SUGARS AS PERCENTAGE OF FRESH WEIGHT IN VARIOUS ROOT TISSUES OF *PANDANUS VEITCHII* GROWN IN NUTRIENT SOLUTIONS AND CONTAINING EITHER AMMONIUM OR NITRATE SALTS AS SOURCES OF NITROGEN

NUTRIENT SOLUTION	SUGAR FRACTIONS	MAIN ROOT TISSUES						ROOTLET
		PROXIMAL		INTERMEDIATE		TERMINAL		TISSUE
		CORTEX	STELE	CORTEX	STELE	CORTEX	STELE	
Ammonium nitrogen	Total .....	1.20	2.60	0.48	1.88	0.52	2.35	0.02
	Reducing .....	1.10	0.69	0.48	0.96	0.52	2.11	0.02
	Sucrose ...	0.10	1.91	0.00	0.92	0.00	0.24	0.00
Nitrate nitrogen	Total ....	0.90	3.31	.....	.....	0.35	1.64	0.04
	Reducing .....	0.90	1.10	.....	.....	0.35	1.06	0.04
	Sucrose ..	0.00	2.21	.....	.....	0.00	0.58	0.00

creased rate of translocation to the rootlets. With respect to the location in the cortex the quantities of reducing sugars increase from the terminal to the basal end of the main root. A comparison of the distribution of re-

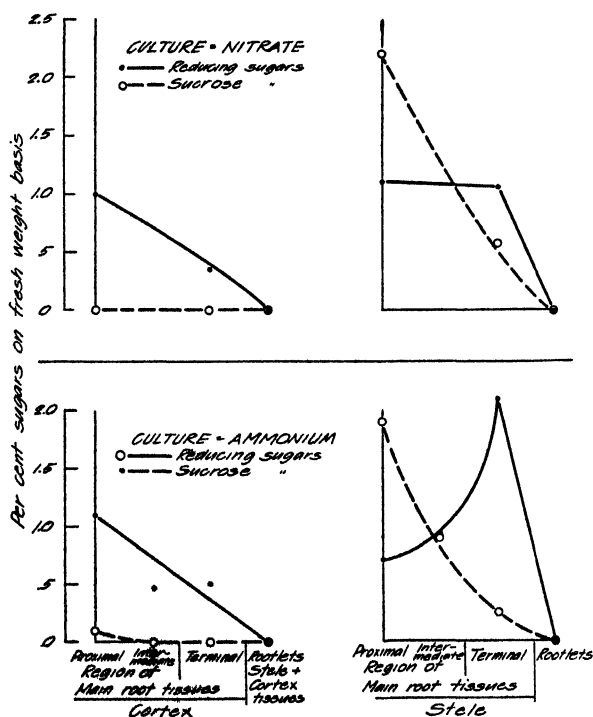


FIG. 7. Distribution of reducing sugars and sucrose in the rootlets and cortex and stele tissues of the terminal, intermediate, and proximal sections of the main root of *P. veitchii* grown in ammonium- or nitrate-solution cultures.

ducing sugars in the stele and cortex shows that within the same fraction of the root there is an indirect proportionality, in that the amounts of reducing sugars in the stele are high and those in the cortex are low. The rootlets contain only traces of these sugars.

3. Sucrose is limited almost exclusively to the stele, the cortex containing only traces or none. The quantities of sucrose in the stele increase from the terminal towards the basal end of the main root, apparently indicating that the conversion of reducing sugars to sucrose is a highly specialized function of the stele and that with a further upward movement of the reducing sugars into the stele their conversion into sucrose is more complete.

NITROGEN AND SUGAR DISTRIBUTION GRADIENTS.—In the three fractions of the main root the quantities of sugars are comparatively enormous as compared to those in the lateral rootlets. The exceedingly small amounts found in the rootlets may be attributed either to a rapid utilization or to a decreased rate of transport from the main root. The amounts of soluble organic nitrogen are almost as great in the rootlets as in the main root, although they should seemingly have been higher owing to their closer contact with the source of inorganic nitrogen. The amounts of insoluble nitrogen of the rootlets are also considerably smaller than those of the main root tissues, which should have been greater in the former than in the latter on the basis of cellular senility and vacuolation. Adequate reasons to explain all these differences cannot be offered without additional information. It is possible, however, to explain certain of the differences on the basis of anatomical variations between the tissues of the main root and those of the rootlets. The ratio of the area or volume of stele to cortical tissues in different fractions of main roots varying in diameter between 10 and 35 mm. ranges from 0.425 to 3 whereas in those rootlets varying in diameter from 2.5 and 10 mm. the range is from 0.114 to 0.425. A more comprehensive idea of the

TABLE VI

AREA IN SQUARE MILLIMETERS AND PERCENTAGE OF STELE AND CORTX TISSUES IN CROSS SECTIONS OF ROOTS OF DIFFERENT DIAMETERS

ROOT CROSS SECTION		STELE		CORTX	
DIAMETER	AREA	AREA	PERCENTAGE OF TISSUE	AREA	PERCENTAGE OF TISSUE
<i>mm.</i>	<i>mm.<sup>2</sup></i>	<i>mm.<sup>2</sup></i>	<i>%</i>	<i>mm.<sup>2</sup></i>	<i>%</i>
37.0	1076.0	858.0	79.6	218.0	20.4
13.0	133.0	50.0	37.5	83.0	62.5
8.0	50.0	12.5	25.0	37.5	75.0
5.0	19.7	3.2	15.8	16.5	84.2
2.5	4.9	0.5	10.0	4.4	90.0

area occupied by stele and cortical tissues in a cross section of roots of different diameters may be obtained from table VI, where such areas are recorded in square millimeters, and also from figure 8 where the areas occupied have been plotted on a percentage basis.

Our detailed analytical data of the stele and cortical tissues of the main root indicate, with a few minor exceptions, that the amounts of nearly all fractions of nitrogen in the stele are considerably higher than in the cortex. One may conclude, on the basis of the analytical and anatomical data, that the relatively low values of the different fractions of nitrogen in the rootlets are due, in a great measure, to a predominance of cortex over stele. With

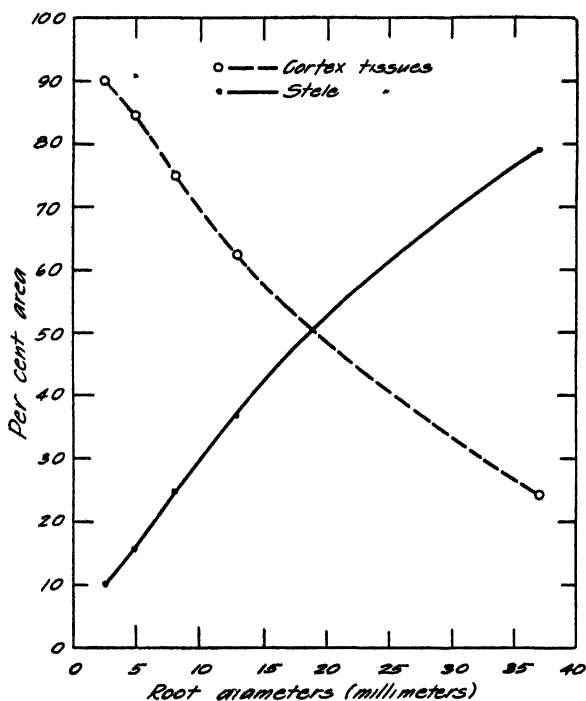


FIG. 8. Distribution in percentage of the area of cortex and stele tissues in the roots of *P. veitchii* varying in total diameter.

respect to the distribution of sugars in the tissues of the rootlets very little can be said because in the compound samples of both stele and cortex these substances were found only in traces.

There are decided gradients of both nitrogen and sugars between the distal and proximal regions of the main root. The quantities of inorganic and soluble organic nitrogen and proteins are greater in the terminal than in the intermediate fractions of the root. The amounts of reducing sugars are high in the intermediate and low in the terminal tissues of the cortex of

the main root, but these values are reversed in the stele of the same regions because of the conversion of reducing sugars to sucrose.

### Discussion

Ammonium assimilation by the roots of *Pandanus veitchii* is very rapid if not instantaneous. Very small quantities of ammonium are nearly always present in the tissues and probably result in most cases from hydrolyzed proteins. At least it is present in comparable concentration even though there is no external nitrogen supply available (figs. 2, 3). The evidence that ammonium is assimilated instantaneously can be best obtained from root exudates, as the tracheal sap under such conditions contains very little if any proteins or materials of injured or crushed cells which may serve as sources of ammonium from hydrolyzed proteins.

The immediate stable products of ammonium assimilation, which are found in appreciable amounts in the tissues, are amino acids, glutamine, and asparagine. The various biochemical reactions which have preceded the synthesis of these compounds are unknown. Glutamine has been found by CHIBNALL and WESTALL (7), VICKERY, PUCHER, and CLARK (79) to be produced in great amounts in plant tissues after ammonium assimilation. In the present case the amounts of glutamine obtained in the exudate and in the tissues of the roots were relatively small indicating that either the concentrations of ammonium supplied in the nutrient solution were comparatively low or that the metabolic mechanism of *Pandanus veitchii* is such as to produce more amino instead of amide nitrogen from ammonium, a condition observed by SCHWAB (63) in many plants.

Nitrate is not assimilated as readily as ammonium by the root tissues of *Pandanus veitchii* as comparatively great amounts pass through the tissues entirely unaltered and are found in the exudate. The relatively slower rate of nitrate assimilation, as compared with that of ammonium, may be due to the various intermediary but highly necessary reduction reactions preceding assimilation, the velocity of which varies considerably in different species and under different conditions. With certain plants nitrate may be assimilated, under highly favorable conditions, entirely in the roots whereas with other plants nitrate assimilation may be conducted mostly in the leaves (20, 35). With respect to *Pandanus veitchii*, where oxygen supply (due to aeration) and carbohydrates were plentiful, the decreased rate of assimilation may be attributed to some inherent causes or to factors of which we have very little comprehension. Experimental results (unpublished) indicating a very low rate of nitrate assimilation by pineapple roots and a higher one by the leaves of the same plant have been obtained by the writers. Woo (81), as well as others, has obtained similar evidence for other plants.

The amounts of asparagine formed during the assimilation of nitrate, particularly in the tissues of the stele, are considerably greater than those obtained during ammonium assimilation. Whether the oxygen atoms in the nitrate molecule are responsible for the formation of greater amounts of asparagine or not, remains to be shown by additional experimentation.

Ammonium more than nitrate, owing to its greater degree of assimilability (7, 9, 13, 26, 64, 65), produces larger quantities of such fractions of soluble organic nitrogen as mono-amino and basic nitrogen at the terminal region of the main root. The quantities of similar fractions of soluble organic nitrogen appear to increase more in the intermediate tissues of the main root of plants grown in nitrate than in the comparable region of those grown in ammonium cultures. This condition may be due to the gradual assimilation of greater amounts of nitrate toward the proximal end of the root where sucrose is more plentiful.

The different fractions of protein or insoluble nitrogen are greater in the roots grown in nitrate than in ammonium cultures. However, no explanation thoroughly satisfactory and unbiased can be offered of this phenomenon. The writers' tentative opinion is that plants grown in nitrate maintain, even under optimal conditions, a relatively low rate of amino acid synthesis. Carbohydrates (and possibly other substances, whatever these may be) essential for protein synthesis are therefore available in sufficient amounts, and proteins are produced regularly and without interruption at all times. But with plants grown in ammonium, under favorable conditions, there is a high rate of organic nitrogen synthesis. In consequence, carbohydrates (and possibly other substances, whatever they may be) essential for protein synthesis are depleted very rapidly in the formation of amino acids. In the absence of these substances the rate of synthesis of proteins decreases and the more readily formed products of soluble organic nitrogen accumulate in the tissues in great amounts. This suggestion does not mean that the synthesis of proteins from nitrates is direct nor does it contradict a long accepted theory (27) that proteins are formed from amino acid or amide nitrogen. BARTON-WRIGHT and McBAIN (5) have observed a somewhat similar condition in the potato and advanced the theory that protein nitrogen is formed from nitrate nitrogen by the direct conversion of the latter first into proteose nitrogen and then into protein. DIKUSSAR (13), in his studies on the assimilation of nitrite nitrogen by higher plants, has presented evidence in which he compares values of absorbed nitrogen from nutrient solution containing nitrite, nitrate, or ammonium, showing that with small amounts of ammonium, nitrate, or nitrite in the culture solution very high yields of protein nitrogen in plant tissues can be obtained. With great amounts of ammonium, nitrate, or nitrite in the culture solution the amounts of soluble organic nitrogen in the tissues, compared on a percent-

age basis, increase at a greater rate than those of protein nitrogen. Certain of his values are reproduced in table VII to illustrate this condition.

TABLE VII

TOTAL NITROGEN AND PERCENTAGE OF PROTEIN AND SOLUBLE ORGANIC NITROGEN OF PLANT TISSUES ABSORBED FROM NITRITE, NITRATE, OR AMMONIUM SOLUTION CULTURES CONTAINING DIFFERENT AMOUNTS OF NITROGEN.  
(From DIKUSSAR's table III)

NITROGEN IN NUTRIENT SOLUTIONS	NITRITE			NITRATE			AMMONIUM		
	TOTAL	PRO- TEIN	SOLU- BLE	TOTAL	PRO- TEIN	SOLU- BLE	TOTAL	PRO- TEIN	SOLU- BLE
<i>mg./l</i>	<i>gm.</i>	<i>%</i>	<i>%</i>	<i>gm.</i>	<i>%</i>	<i>%</i>	<i>gm.</i>	<i>%</i>	<i>%</i>
60	0.438	52.0	48.0	0.306	60.8	39.2			
40	0.396	57.0	43.0				0.546	44.3	55.7
20	0.247	72.8	27.2	0.227	66.5	33.5			
10	0.291	75.9	24.1	0.286	74.4	25.6			
5	0.286	74.4	25.6				0.283	76.7	23.3

The experimental values of DIKUSSAR are in harmony with our tentative hypothesis that the degree of assimilability and quantities of inorganic nitrogen coupled with the amounts of other substances essential for amino acid and protein synthesis are factors responsible for the great differences found in the ratio values of protein:soluble organic nitrogen of plants grown respectively in nitrate and ammonium solution cultures.

With respect to substances necessary for protein synthesis the literature points out quite decidedly that an abundance of readily available carbohydrates is highly essential. SUZUKI (66, 67, 68) and MUENSCHER (37) have clearly demonstrated that in the presence of carbohydrates and inorganic nitrogen chlorophyllous plants are able to synthesize soluble organic and protein nitrogen. That besides leaves other plant organs such as roots are able to synthesize proteins either in the presence or absence of light has been demonstrated by MÜLLER-THURGAU (38), IWANOFF (21), and BAMBACIONI (3). IWANOFF, working with the roots of *Brassica napus*, *Daucus carota*, and *Solanum tuberosum*, observed that for the synthesis of protein in the absence of light his plants required a small amount of protein and a great supply of both amides and readily assimilable carbohydrates. BAMBACIONI on the basis of analysis of two sets of plants of *Vicia faba*, *Ricinus communis*, and *Cucurbita pepo*, one grown in plus-nitrogen and the other in minus-nitrogen solution cultures, concluded that all parts of the plant, and particularly the roots, are capable of synthesizing proteins. That carbohydrates are highly essential for protein regeneration in newly forming tissues of plants deprived of nitrogen and obtaining their nitrogen supply from the hydrolyzed

proteins of old tissues has been well demonstrated by PRIANISCHNIKOW (53). There is also undisputable evidence (46, 53) that proteins stored in plant tissues undergo hydrolysis at a much greater rate in the absence than in the presence of carbohydrates.

Yet there are certain cases known where besides carbohydrates certain other factors, of which our knowledge is limited, influence the rate of protein synthesis. LOOSE and PEARSALL (28) claim, from certain experimental evidence they have obtained on *Chlorella*, that the velocity of protein synthesis is five times greater in the light than in darkness though the final equilibrium is similar in both cases if sufficient carbohydrates are available. It is rather unfortunate that these investigators have not reported in their results the amounts of readily assimilable carbohydrate in the tissues of their plants or those in the nutrient solution at the end of the experiment to show additional relationships between carbohydrates and protein synthesis.

Other conditions, and particularly deficiencies of certain mineral elements, although acting indirectly, are capable of reducing the rate of protein synthesis. The work of NIGHTINGALE, SCHERMERHORN, and ROBBINS (47), HARTT (17), RICHARDS and TEMPLEMAN (62) with potassium-deficient plants shows quite conclusively that protein synthesis is retarded in insufficient amounts of potassium. Similar effects have also been reported in plants grown in nutrient solutions deficient in certain other elements besides potassium. The direct effects of available carbohydrates in protein synthesis are well appreciated and easily understood, while the indirect effects of other factors, such as we obtain in mineral deficiencies, complicate this problem to a certain extent.

The results obtained on the distribution of reducing sugars and sucrose in the tissues of the stele and cortex are very interesting. The more or less complete absence of sucrose, and the singular presence of reducing sugars in the tissues of the cortex are indicative of sucrose being a storage and not a transport sugar. Moreover, the inverse proportionality which exists between the quantities of sucrose and reducing sugars in the stele and the greater amounts of sucrose in the proximal than in the terminal tissues indicate that reducing sugars enter the distal portion of the stele through the terminal tissues of the cortex of the main root where they are gradually converted into sucrose. The amounts of sucrose in the proximal region of the stele are higher while those of reducing sugars are lower, showing that the accumulation of sucrose in more mature cells is greater and the conversion of reducing sugars to sucrose more complete. The findings of COLIN (10) on the formation and distribution of sucrose in the sugar beet indicate that, although sucrose is formed in the leaves in sunlight, it is converted into reducing sugars during transportation into the root and there transformed again into sucrose. The experimental evidence of COLIN shows that the quantities

of sucrose diminish from leaves to stem. With respect to the migration of sucrose from the root towards the aerial parts, in two-year-old plants, COLIN states that sucrose leaves the tissues of the root as sucrose but as it approaches the aerial organs it is converted gradually into reducing sugars.

BARTON-WRIGHT and MCBAIN (4) have observed sucrose accumulation in the laminae of diseased crinkle plants, while hexose was abundant in the healthy and starch had fallen to low values in both diseased and healthy plants. Their interpretation of these results, with respect to healthy leaves, is that hexoses are the ultimate products of mostly sucrose and of very little starch hydrolysis and that sucrose is transported out of the leaves to the tubers; whereas with diseased plants sucrose formation is taking place directly and mostly from starch and only to a very small extent from hexose, but that sucrose is the sugar of transport in both cases. Our data do not agree very well with the interpretations of BARTON-WRIGHT and MCBAIN unless we assume an upward transport of sucrose through the tissues of the stele in a manner analogous to that described by MASON, MASKELL, and PHYLLIS (32) with respect to stored nitrogen in which case they state as follows: ". . . the upward movement of stored nitrogen may be an example of upward movement via the phloem in opposition to the downward movement of carbohydrates, but that until more is known as to the conditions determining liberation of stored nitrogen into the tracheae the alternative of upward movement entirely via the xylem cannot be excluded." In a preceding paper MASON and MASKELL (31) state that ". . . when wood and bark are separated movement of sugar takes place through the bark at nearly the normal rate and that the downward transport of carbohydrates occurs in the bark." Our data in table II support the claims of CURTIS (11) and of MASON, MASKELL, and PHYLLIS (32) that there is an upward movement of stored nitrogen through the wood tissues and that reducing sugars are transported through the tissues of the bark as indicated in table V.

The prominent position which sucrose occupies in the stele and its complete absence in the cortex cannot be overlooked. If there is any transport of sucrose at all it will be in an upward direction through the tissues of the stele and probably in the manner indicated by COLIN (10). The tissues of the stele are, from the point of view of sucrose deposition, comparable to the root tissues of the sugar beet as presented by COLIN. In the absence of more information on the nature of transport of sucrose we postpone any further discussion until additional experimental evidence is obtained.

### Summary

A series of experiments was undertaken in order to obtain information on the absorption and assimilation of ammonium and nitrate nitrogen by



analyzing the exudate and the stele and cortex of different fractions of the excised roots of *Pandanus veitchii* grown in ammonium, nitrate, and minus-nitrogen solution cultures respectively. The root tissues, but not the exudate, were also analyzed for reducing sugars and sucrose. The results were as follows:

1. Ammonium is assimilated instantaneously and at a high rate as it enters the root, where it is converted either into amide or amino nitrogen or more gradually into protein.

2. Nitrate is not assimilated as rapidly as ammonium and the amounts of the various fractions of soluble organic nitrogen are not as high, particularly in the terminal root tissues, as those found usually in roots grown in ammonium cultures.

3. The amounts of protein or insoluble nitrogen are greater in the roots of the plants grown in nitrate than in those receiving ammonium nutrition. Our tentative explanation of this condition is that roots grown in ammonium cultures, through the rapid formation of amino acids utilize and exhaust very rapidly the supply of carbohydrates and possibly of other substances essential for protein synthesis. With roots of plants grown in nitrate cultures, the rate of nitrate assimilation and synthesis of amino acids being comparatively low, carbohydrates and other substances essential for protein synthesis are not depleted but are always present in sufficient amounts to promote protein synthesis, the ultimate stage of nitrogen metabolism.

4. Organic nitrogen, newly synthesized in the roots, either from ammonium or nitrate, is transported through the tissues of the stele to the proximal regions of the root, following the path of water and mineral salt conduction in the roots. The channels which the hydrolytic products of stored nitrogen follow are the same, if our interpretation of the results obtained in minus-nitrogen nutrient cultures is correct. Certain of these studies made on root exudates indicate that, except for a great difference in the amounts of soluble organic nitrogen between plus-nitrogen and minus-nitrogen cultures, no other lines of demarcation can be drawn with our present analytical technique between newly synthesized and stored fractions of soluble organic nitrogen.

5. No proteins, in any appreciable quantity, were found in the exudate of roots, indicating that assimilated nitrogen is translocated as soluble organic and not as insoluble organic or proteinaceous nitrogen.

6. The distribution of reducing sugars and sucrose in the tissues of the stele and cortex shows that sucrose is present in great amounts in the stele but is lacking almost completely in the cortex. The data also suggest that reducing sugars, possibly, enter the tissues of the stele through the terminal tissues of the cortex of the main root. The amounts of reducing sugars in the stele are greatest at the point of entrance or terminal region, but de-

crease in the upper regions of the stele. At different levels along the root there is in the stele an indirect relationship between the amounts of reducing sugars and sucrose. In the terminal portion of the stele, which may serve as paths of entrance, reducing sugars are higher than sucrose, but in the proximal region of the main root, sucrose increases very rapidly in the stele, whereas reducing sugars decrease in about the same ratio, apparently indicating that there is a gradual but rapid conversion of reducing sugars to sucrose.

7. The data suggest that reducing sugars are the sugars of downward transport and that sucrose is stored in the stele. If sucrose ever enters the transport stream in the roots of *Pandanus veitchii* it may follow a course similar to that in the sugar beet, that is, an upward instead of a downward direction. The tissues of the stele of the roots of *Pandanus veitchii*, on account of their ability to synthesize and store sucrose, are comparable to the root tissues of the sugar beet, as discussed by COLIN.

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# EFFECT OF ENVIRONMENTAL FACTORS UPON THE COLOR OF THE TOMATO AND THE WATERMELON<sup>1</sup>

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(WITH SEVENTEEN FIGURES)

## Introduction

A contribution to the knowledge of the physiological mechanism involved in the production of color in fruits of commercial value is of considerable advantage and usefulness at the present time. A premium is always paid for desirably colored products, both in the canning and in the fruit and market gardening industries. The natural color of ripe fruits may be due to pigments of very diverse nature, produced by the protoplast either in the living plastid, in the cell sap, or deposited in the cell wall. The plant physiologist is particularly interested in the biochemical changes which are responsible for the development of these varied pigments, the conditions under which they are produced, and their ultimate fate in the animal body. At the present time the layman is fast becoming familiar with the fact that certain of these yellow plant pigments are precursors of vitamin A and play an important rôle in human nutrition.

The chlorophyll of higher plants is produced in special organs of the living cell called chloroplasts. According to ZIRKLE (63) first mention of these "green granules" was made by COMPARETTI (13) in 1791. The living nature of these bodies, however, was not established until almost a century later when SCHIMPER (51) proved that they arise by division of preexisting structures and gave them the name "plastids." The pigments of the green leaf were first extracted and given the name "chlorophyll" by PELLETIER and CAVENTOU (50) in 1818, but it was not until 1864 that the first separation of the chlorophyll complex into green and yellow components was made by STOKES (55). In a paper before the Royal Society he stated: "I find the chlorophyll of land plants to be a mixture of four substances, two green and two yellow, all possessing highly distinctive optical properties. The green substances yield solutions exhibiting a strong red fluorescence; the yellow substances do not." It is to this man that science owes the discovery of the still widely used method of partition between two immiscible solvents. He stated definitely, "For convenience and rapidity of manipulation, especially in the examination of very minute quantities, there is no method of separation equal to that of partition between solvents which separate after agitation." Unfortunately, this discovery was overlooked by

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plant physiologists and did not come into general use until after BORODIN (11) isolated two different types of crystals from the yellow constituent. In 1906 TSWETT (57) introduced the chromatographic method for the separation of the component pigments, which also is widely used today. Furthermore, it was TSWETT who introduced the term "carotenoid" and proposed grouping the several yellow pigments found in leaves under this name. ARNAUD (9) some years previously had investigated the yellow pigment accompanying chlorophyll and discovered that it was identical with the carotene isolated from the carrot (*Daucus carota* Linn.) by WACKENRODER (58) in 1831. ARNAUD first established the hydrocarbon nature of the pigment and proposed a relationship between the yellow pigments and chlorophyll, suggesting a rôle for them similar to that of hemoglobin. It is interesting to note that recently JAVELLIER, ROUSSEAU, and EMERIQUE (28) examined one of ARNAUD's preparations sealed in a tube of hydrogen and found it to possess biological activity after a period of forty years.

In 1913 MONTEVERDE and LUBIMENKO (44) presented a spectrophotometric method for the estimation of plastid pigments and studied their transformation in living plant tissue. In the same year WILLSTÄTTER and STOLL (61) applied the method of splitting a complex molecule into cleavage products. By purification and further study of these products they were able to reconstruct the chlorophyll molecule and by this means obtained proof of its constitution. During the course of these investigations WILLSTÄTTER and MIEG (60) obtained the two yellow pigments as secondary products in extracting chlorophyll from nettle leaves and established their empirical formulae. The observation that carotenoids may act as oxygen carriers or absorbers led WILLSTÄTTER to introduce this concept into the photosynthesis mechanism, as a means of reducing chlorophyll b and keeping the a and b components in equilibrium. In 1927 ZECHMEISTER (8) showed by catalytic hydrogenation that carotene contained eleven ethylenic linkages and was a bicyclic compound. Lycopene was found by the same investigator to contain thirteen double bonds and to be an unsaturated aliphatic molecule. Lycopene was first discovered in the fruit of the tomato (*Lycopersicon esculentum* Mill.) by MILLARDET (40) in 1876, although it did not receive the name lycopene until 1903 when SCHUNCK (52) presented a paper before the Royal Society of London. WILLSTÄTTER and ESCHER (59) isolated this red pigment from Italian tomatoes in 1910 and suggested its hydrocarbon nature. Today both carotene and lycopene are recognized as polyene hydrocarbons and PALMER (49) suggests a new system of nomenclature for the entire group. Furthermore, KUHN (32) has shown that the color of certain carotenoid compounds depends upon a chain of more than four conjugated double bonds and presented a method of separation of carotenoid pigments which has been developed further by MILLER (41). The fact that the smell

of violets was apparent on the oxidation of carotene led KARRER (3) to believe the odor due to the presence of  $\beta$ -ionone rings. Subsequent investigation proved the symmetrical structure of carotene and that both ends of the hydrocarbon chain were cyclic and identical. Lycopene was found to add thirteen molecules of hydrogen, forming perhydrolycopene and by a series of reactions lycopene was shown to be a symmetrical acyclic hydrocarbon carrying thirteen double bonds, eleven of which are conjugated. The deep color of lycopene adds weight to KUHN's (33) observation on crocetin and bixin that deep color requires more than four conjugated double bonds. Furthermore, KARRER accomplished the difficult synthesis of perhydrovitamin A and then was able to present the structural formula of vitamin A. Previous to this time OSBORNE and MENDEL (48) and McCOLLUM and DAVIS (38) had found the A factor to be present in cod liver oil and in butter. STEENBOCK (54) brought out the fact that only products containing certain yellow pigments had vitamin A potency. DRUMMOND (17) repeated the work using pure carotene and obtained negative results due to the solvent used. It remained for EULER (2) and KARRER (3) independently of each other, to harmonize these apparently discordant results and to prove  $\beta$ -carotene to be the parent substance which is converted to vitamin A by the animal body. Numerous contributions by these and other investigators have shown the existence in plants of a series of polyene hydrocarbons, a series of polyene alcohols, and several polyene carboxylic acids. The carotenoid pigments are of wide distribution in nature and are responsible for a great number of colors in all plant organs. They are known to exist together and in combination with other organic pigments, so that fruit or flower color is likely to consist of a pigment complex, variation being due to the balance between the various pigments involved.

The marked effect of temperature upon the production of lycopene was first discovered by DUGGAR (18) in 1913. This remarkable contribution showed conclusively that a temperature of 30°–37° C. clearly inhibited the development of lycopene both in detached fruit and in fruit growing on the vine. The factors for reddening were not destroyed by the high temperature, for upon return to a temperature of 20° C. lycopene formation proceeded rapidly. Lycopene formation follows the destruction of the chlorophyll but certain other changes which take place within the cells remain unknown. Lycopene formation, however, does not necessarily follow decomposition of chlorophyll as HARVEY (27) has recently shown. The chemical reactions and the relationship between the pigments, the source from which the pigments are derived, the physiological importance of each component, are questions which remain little understood and for which quest is being carried on throughout the world. Lycopene formation occurs only at temperatures above 10° C. and below 37° C. Chlorophyll decomposition

requires a temperature above 15° C. and is removed less rapidly at 40° C. than at 24° C. In addition, HARVEY (26) has shown that chlorophyll decomposition by ethylene can occur only when the cells are in an active metabolic state. The presence of fungi, slight mechanical injuries, or certain other conditions prevent the balancing action of ethylene. Therefore, it is likely that the decomposition of chlorophyll does not take place by a single enzyme mechanism, but that the whole actively metabolic system of the cell is required. The same is indicated for lycopene formation.

Extensive reviews of the literature are found in monographs by KARRER (3), ZECHMEISTER (8), LEDERER (4), MAYER (5), BERGMANN (1), and PALMER (6).

### Determination of color

The subject of color is a most fascinating one for scientist and layman alike. Man has been color-conscious for a long time, the word itself being very old. Like many old words, the word "color" has been used to convey different meanings. As a household word, its meaning is vastly different from its use by the physicist.

Color depends on the constituents of white light which are not absorbed but which are reflected. Color sensations in the normal individual depend on the responses of the retina of the eye to different wave lengths of light. If the light has one definite wave length, the color produced will have a pronounced hue. When Sir ISAAC NEWTON passed white light through a glass prism, he obtained a continuous spectrum containing all the spectral hues, which may be described as red, orange, yellow, green, blue, and violet. However, NEWTON recognized their objective physical meaning, saying plainly, "The rays, to speak properly, are not coloured. In them is nothing else than a certain power or disposition to stir up a sensation of this or that colour. . . . So colours in the object are nothing but a disposition to reflect this or that sort of rays more copiously than the rest." NEWTON also knew that any color could be matched by mixing the component blue, green, and red portions in proper proportions. If the primary spectral colors, *i.e.*, light of wave lengths of 460, 530, and 650 mμ be mixed in varying proportions, any hue whatsoever can be produced. The visible spectrum is arbitrarily divided into six broad regions (fig. 1), but the color of the spectrum varies continuously throughout its length. The physical difference between red, green, or blue is one of wave length. The unit commonly used for specifying this difference is the millimicron. Table I shows the approximate wave lengths of these six broad regions.

In the analysis of color the eye is not a good analytical instrument. It gives a confused sensation owing to the inability clearly to distinguish between the three components of color, particularly brightness and purity. When a color is measured spectroscopically, such confusion is eliminated.

**TABLE I**  
SPECTRAL REGIONS IN TERMS OF WAVE LENGTH

	<i>mμ</i>
Violet .....	400-450
Blue .....	450-500
Green .....	500-570
Yellow .....	570-590
Orange .....	590-610
Red .....	610-700

If the light from a reflected surface is measured by a colorimetric method, the eye perceives only those wave lengths to which it is sensitive. Different eyes are sensitive in different degrees to different wave lengths. In such a procedure different observers may obtain different results. Also, when a color is observed under varying quality of illumination, the sensation produced under each light will be different. Colorimetric measurements, therefore, are not dependent on the light source alone, but also on the idiosyncrasies of human vision. Spectrophotometry, on the other hand, provides a method for completely specifying the stimulus of color independent of material color standards, or of abnormalities of the observer's vision and other personal eccentricities. The color of a reflecting surface can be determined with sufficient precision, and the analysis of any color can be plotted in the form of a spectrophotometric curve, as in figure 1. Thus, two or more colors may be compared by comparing their curves, each of which constitutes a permanent record of the color and does not require the maintenance of a sample of the color.

The color of green tomato fruits is due to the complex chlorophyll mixture within the chloroplast. The fact that yellow pigments accompany chlorophyll has been known since the fundamental work of STOKES in 1864. Upon the decomposition of chlorophyll at temperatures from 30°-37° C., carotenes, xanthophylls, and certain yellow pigments give the tomato its characteristic color. If the temperature during the ripening period is held below 30° C., unknown changes take place within the actively metabolic cells, resulting in the formation and accumulation of lycopene, the carmine-red isomer of carotene. The color changes due to the physical mixture of the various pigments can be satisfactorily determined by spectrophotometric reflection measurements. Thus, optics furnishes the plant physiologist with an accurate method of color measurement, and enables him to interpret color changes owing to the fact that there are two or more pigments present in physical mixture rather than in chemical combination.

In this work reflection measurements from the surface of the fruit were taken with a Keuffel and Esser direct-reading spectrophotometer. The re-

reflection measurements thus obtained were plotted in the form of a spectrophotometric curve and also converted into terms of the red, green, and violet excitation values. From these values the dominant wave length and percentage of purity were read from a copy of the chart as published in the report of the committee of the Optical Society of America (56) on colorimetry for 1920-21. The percentage of relative brightness was also computed so that the colors of fruits are given in numerical expression of the three attributes of color, namely, hue, saturation, and brilliance.

In describing the color stimulus in terms of the psychological sensation which it produces, all of these attributes of color must be considered. The Committee of the Optical Society of America (56) suggests the following definitions: "Brilliance is that attribute of any color in respect of which it may be classed as equivalent to some member of a series of grays ranging between black and white." Brilliance is expressed as percentage of relative brightness. The percentage of brightness of a color defines that proportion of the total amount of white light falling upon it that the color is capable of reflecting or transmitting. All colors except black exhibit brilliance. The brightness of absolute black is zero, that of pure white 100 per cent.

"Hue is that attribute of certain colors in respect of which they differ characteristically from the gray of the same brilliance and which permits them to be classed as reddish, yellowish, greenish, or bluish." Hue is expressed as dominant wave length.

"Saturation is that attribute of all colors possessing a hue, which determines their degree of difference from a gray of the same brilliance." Saturation is expressed as percentage of purity. All colors which exhibit a hue must also exhibit a saturation. Grays have zero percentage purity. Saturation determines the degree in which a color possesses hue; thus, the percentage purity defines how red or how green, or how yellow a color is. Purity and chroma are synonymous terms.

For example, spectrophotometric reflection measurements show the color of a ripe tomato as having a brightness of 11.8 per cent. a dominant wave length of 609  $\mu$ , and a purity of 24.5 per cent. The spectrophotometric curve is presented in figure 1; the dominant wave length shown by the vertical line falls in the orange-red region of the spectrum, and the observer pictures an attractive red fruit. A red and a pink may have the same dominant wave length but will differ in percentage of purity; pink, being an unsaturated red, will have a lower percentage of purity. A pure spectral red has a purity of 100 per cent. On the other hand, the red will have a lower percentage of brightness owing to the lesser proportion of white in it. Where the curve is an approximate straight line parallel to the abscissa, the color is said to be gray. The horizontal line of zero relative energy will represent black, whereas the line of 100 relative energy will represent white.

Curve 2 is a spectral reflection curve of a Marglobe tomato ripened at 32° C. and which appears to the eye as a brilliant yellow. In numerical terms the color of this fruit would be described as possessing a brightness of 33.5 per cent., a dominant wave length of 581.5 m $\mu$ , and a purity of 59 per cent. Curve 3 is plotted from reflection measurements taken from a typical Marglobe tomato picked in the so-called "mature green" stage. The color of this fruit is described as having a brightness of 32.9 per cent., a dominant wave length of 570 m $\mu$ , and a purity of 53 per cent. Such a color indicates a yellowish green in which the green components of chlorophyll to a large extent mask the yellow ones.

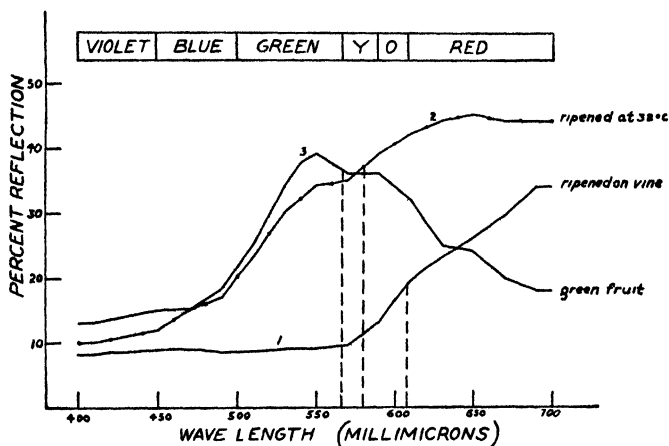


FIG. 1. Color analysis of Marglobe tomatoes.

This method of color analysis was chosen because the spectrophotometer is an instrument of precision by means of which the attributes of color can be measured accurately. As previously pointed out, the eye is not an analytical instrument, and, with advance in color measurement, spectrophotometers will undoubtedly become more readily available in the future.

### Effect of temperature

#### EFFECT OF TEMPERATURE UPON DEVELOPMENT OF COLOR IN TOMATO

In an attempt to ripen quickly some tomato fruits which were gathered green to avoid losing the fruits from frost DUGGAR (18) made the discovery that those fruits ripened at a temperature of 20° C. in the dark for seven days had a much redder color than those stored in an incubator at a temperature of 35° C. for an equal length of time. This observation led to a series of carefully planned and executed experiments on greenhouse-grown Earliana tomatoes. Later similar experiments were conducted on species of *Capsicum* and *Momordica*. In all cases fruits stored at temperatures above 30° C. and

below  $37^{\circ}\text{C}$ . showed very little production of lycopene and were distinctly yellow in color. The same results were obtained when only half-grown fruits were tried and also when the experiments were extended to cover fruits developing on the vine. These facts led DUGGAR to conclude that the optimum temperature range for lycopene formation is narrow and that its optimum coincided with the optimum for growth or perhaps was a few degrees lower. Furthermore, the factors for reddening were not destroyed by a temperature of  $37^{\circ}\text{C}$ ., for when such fruits were returned to favorable temperature of  $20^{\circ}\text{C}$ ., lycopene development proceeded and in four or five days the yellow fruits became red. EULER, KARRER, KRAUS, and WALKER (20) confirmed the observation that temperatures above  $30^{\circ}\text{C}$ . produce tomato fruits which are yellow in color. They suggest that the yellow pigment so induced is not

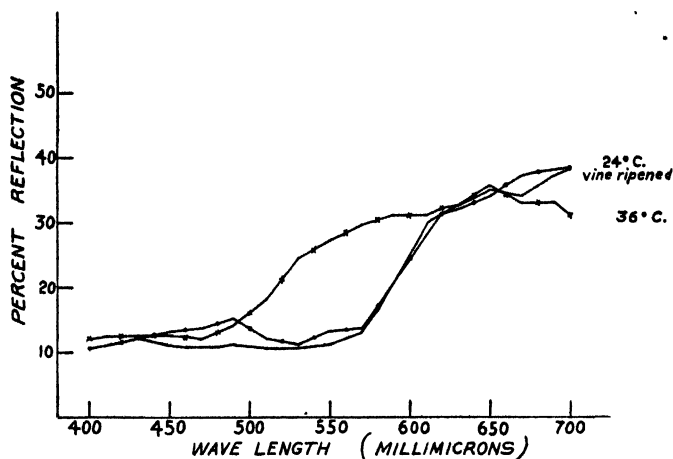


FIG. 2. Effect of temperature ( $24^{\circ}$ ,  $36^{\circ}\text{C}$ .).

of a carotenoid nature but is possibly a flavone or flavinol. However, the last named authors make the clear assumption that lycopene formation is an enzymatic process and that lycopene synthesis does not occur at temperatures above  $30^{\circ}\text{C}$ . because of the destruction of the unknown enzyme.

In the experimental work herein reported the author used Bonny Best and Marglobe varieties of tomatoes. The fruits were picked green and selected for uniformity in size and in approximate age from blossoming time. The fruits were then stored for twelve to twenty-four hours at  $20^{\circ}\text{C}$ . and again finally selected for experimental work. The fruits were placed in darkness in constant temperature cases of two and one-quarter cubic feet capacity and the temperatures were held at  $24^{\circ}$ ,  $28^{\circ}$ ,  $32^{\circ}$ ,  $36^{\circ}$ , and  $40^{\circ}\text{C}$ . ( $\pm 1^{\circ}\text{C}$ .). Reflection measurements were taken with a Keuffel and Esser spectrophotometer at the close of a seven-day period in the case of series 1 and 4, and at the end of a nine-day period in series 2 and 3. Series 2, 3, and 4 served also

as checks in the work on the effect of ethylene, of varying concentration of oxygen, and of light respectively. Moisture was supplied by evaporation from open dishes in each constant temperature case. Reflection measurements were taken from three representative tomatoes in each case. To obtain a flat surface for reflection a section of the pericarp wall 22 mm. in diameter was removed with a sharp cork borer and this section mounted in the 25-mm. cell which accompanies the instrument. Such a section when flattened filled the cell and gave a uniform reflection surface. Measurements were recorded at 20-m $\mu$  intervals from 450 to 690 m $\mu$  respectively. Since the analytical work requires a reading at 10 m $\mu$  intervals from 400 to 700 m $\mu$  respectively, assumed readings were interpolated or extrapolated as necessary. However, the sensitivity of the eye below 450 and above 690 m $\mu$  is so

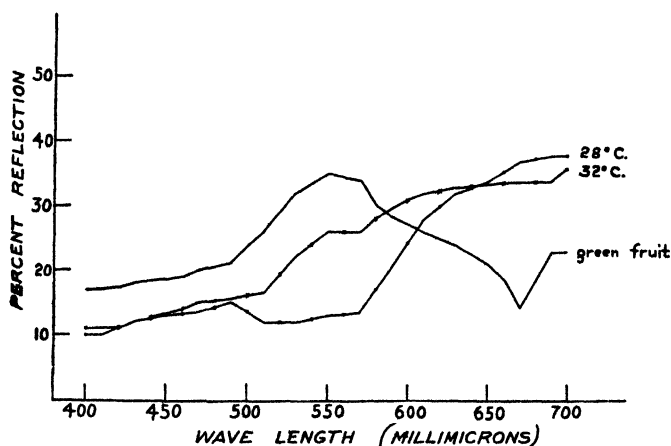


FIG. 3. Effect of temperature (28°, 32° C.).

low that an assumed value is sufficiently accurate. The percentage of reflection was recorded, and the red, green, and violet sensations and the relative luminosity as compared to the luminosity of noon-day sun were calculated with the aid of a special Keuffel and Esser slide rule. From these data the brightness, dominant wave length, and purity of each sample were calculated and are presented in table II.

The most remarkable observation in respect to the effect of temperature (fig. 4) is the great difference in percentage of brightness and percentage of purity of samples ripened at 24° and 28° C. as compared with those ripened at 32° and 36° C. This difference is even more pronounced when dominant wave length is considered. Fruits ripened at 32° and 36° C. show a dominant wave length of from 500 to 588 m $\mu$ , appearing bright yellow, while those ripened at 24° and 28° C. show a dominant wave length of from 595.5 to 609.5 m $\mu$  and appear a deep orange-red to the eye. Spectral reflection curves are presented in figures 2 and 3.



When the bright yellow fruits ripened at 32° and 36° C. are returned to a temperature 20° C., they rapidly turn orange-red and show a dominant wave length of 592.5 to 597.0 mμ. After four days at 20° C., such fruits are as red as normal fruits ripened on the vine and show a dominant wave length of from 600 to 610 mμ.

A second remarkable effect of temperature of great importance is the fact that both Bonny Best and Marglobe varieties of tomatoes when ripened at 40° C. do not turn yellow but remain green, i.e., chlorophyll decomposition is prevented. Frequently brown spots of various size and shape develop after four or five days and such fruits in which normal metabolic activity is at least seriously checked are quickly attacked by bacteria and other fungi. Fruits ripened at 40° C. for seven days show a dominant wave length of

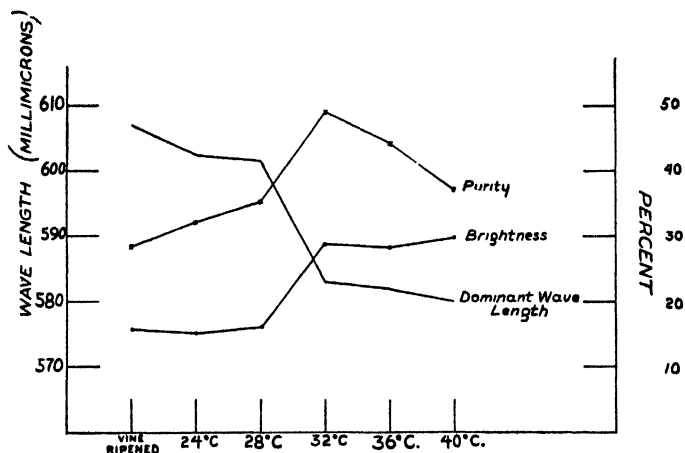


FIG. 4. Effect of temperature on purity, brightness, and dominant wave length.

578.5 mμ, a brightness of only 22.96 per cent., and a purity of 22.5 per cent. and appear a dull green to the eye. This low purity as compared with normal green fruit indicates unsaturation, i.e. a gray-green of low brilliance, having a larger proportion of white admixed. When these fruits ripened at 40° C. are transferred to a temperature of 20° C., they do not produce lycopene as do the fruits ripened at 32° or 36° C. but very quickly undergo internal breakdown. Such fruit are particularly subject to attack by *Bacillus carotovorus*, *Phoma* sp., *Rhizopus nigricans*, and other saprophytic organisms. The normal metabolic activity of such fruits is seriously interfered with, resulting in the more or less complete disorganization of the plasma structure. With this disorganization the complete destruction of the whole cellular mechanism for lycopene formation takes place. This great difference which is produced when the temperature is changed from 36° to 40° C. is remarkable and with other previously mentioned reasons indicates that

TABLE II  
EFFECT OF TEMPERATURE UPON PRODUCTION OF COLOR IN TOMATO

SERIES 1 + 4-9 DA., 2 + 3-7 DA.	BRIGHTNESS				DOMINANT WAVE LENGTH				PURITY			
	SERIES 1	SERIES 2	SERIES 3	SERIES 4	SERIES 1	SERIES 2	SERIES 3	SERIES 4	SERIES 1	SERIES 2	SERIES 3	SERIES 4
Vine ripened fruit .....	16.33	17.68	16.93	11.80	610.0	605.0	605.0	609.0	25.0	32.0	31.5	24.5
Green fruit .....	29.91	30.45	26.26	31.21	565.0	570.0	577.5	562.0	35.0	40.0	37.0	40.0
24° C. ....	16.93	16.74	15.73	11.38	608.0	600.0	600.0	602.5	23.0	30.0	32.5	33.0
28° C. ....	15.89	17.91	16.06	14.17	609.5	600.0	595.5	604.0	22.0	35.0	46.0	38.5
32° C. ....	25.36	22.32	30.81	36.83	582.5	588.0	582.0	580.0	45.5	42.5	57.0	51.0
36° C. ....	25.69	26.02	33.39	28.31	582.0	580.0	583.5	582.5	50.0	32.0	51.2	44.0
40° C. ....	22.96	33.65	32.74	30.21	578.5	580.0	581.0	579.0	22.5	39.0	42.0	45.0
32° C. + 2 da. 20° C. ....	19.02	.....	.....	.....	597.0	.....	.....	.....	29.00	.....	.....	.....
32° C. + 4 da. 20° C. ....	16.63	.....	.....	.....	605.0	.....	.....	.....	30.00	.....	.....	.....
36° C. + 2 da. 20° C. ....	18.51	.....	.....	.....	592.5	.....	.....	.....	42.00	.....	.....	.....
36° C. + 4 da. 20° C. ....	17.35	.....	.....	.....	600.0	.....	.....	.....	32.00	.....	.....	.....

lycopene formation is not a simple enzymatic change but, like the decomposition of chlorophyll, is an internal change which can take place only in actively metabolic cells, and emphasizes the physiological inequalities of different temperature ranges.

#### EFFECT OF TEMPERATURE UPON DEVELOPMENT OF COLOR IN WATERMELON

Both KARRER (3) and ZECHMEISTER (8) state that lycopene is the red pigment in the watermelon (*Citrullus vulgaris* Schrad.). A series of experiments was planned to determine the effect of temperature upon lycopene production in this fruit. It was found to be impossible to hold green watermelon fruits at temperatures of 24° to 40° C. for more than a few days. Fruits which were removed from the vine were quickly attacked by saprophytic fungi and were reduced to a decayed mass in a relatively short time. One lot of melons, variety Winter Queen, were successfully held at 24°, 28° and 32° C. for fifteen days.

The spectrophotometric data for these fruits, as seen in the accompanying table, show that in fifteen days the fruits have undergone very little change regardless of the temperature at which they were held. This difference in behavior between the watermelon and the tomato led to further attempts to ripen watermelon fruits under temperature control. All other attempts to artificially ripen watermelons off the vine gave negative results because of fungus attack. Removal of fruits with ten to fifteen feet of vine, trial feeding of immature melons with glucose, and several other attempts proved futile. However, it was deemed practical to move temperature equipment to the field. Three fruits of variety Arikara were held at controlled temperature for nineteen days. One fruit was kept in the dark at 18° to 22° C., another was kept at 33° to 37° C. in the light, and a third melon was held in the same higher temperature case but in the dark. The color analyses of such fruits show close agreement, brightness varying from 14.62 to 16.43 per cent., dominant wave length from 592 to 595 mμ, and purity from 44 to 50 per cent. (table III).

These data indicate that perhaps the same mechanism for lycopene formation does not exist in the watermelon as in the tomato. A shift in temperature from 20° to 37° C. did not result in the prevention of lycopene formation as in the case of the tomato, the red pigment production continuing without apparent interruption regardless of the temperature. Either lycopene formation proceeds in a different fashion in the watermelon than in the tomato or else we are dealing with a red pigment other than lycopene. BILGER (10) has recently shown that the red pigment from the Japan red pepper differs in chemical composition from lycopene or from the ZECHMEISTER-CHOLNOKY red pigment from paprika, and that this new pigment is not an isomer of carotene. However, BROWN (12), working with the red

**TABLE III**  
**EFFECT OF TEMPERATURE UPON DEVELOPMENT OF COLOR IN WATERMELON**

	BRIGHTNESS	DOMINANT WAVE LENGTH	PURITY
	Variety Winter Queen		
		mμ	
Green fruit .....	31.45	585.0	16.0
24° C. 15 days .....	28.34	589.0	16.0
28° C. 15 " .....	21.06	588.0	23.0
32° C. 15 " .....	31.27	584.5	17.0
36° C. 15 " .....	data not available due to decay		
40° C. 15 " .....	data not available due to decay		
	Variety Arikara (vine ripened)		
18°-22° C. 19 days, dark ....	16.43	592.0	50.0
33°-37° C. 19 days, light .....	14.62	595.0	44.0
33°-37° C. 19 days, dark .....	15.84	595.0	44.0

pigment of the Perfection pimento (*Capsicum annuum*), finds it to be identical with the capsanthin isolated by ZECHMEISTER and CHOLNOKY (62) from the Hungarian paprika. Quantitative data in regard to the identification of the red pigment of the watermelon is needed. Data derived from breeding experiments with both the watermelon and tomato indicate that red is invariably dominant to yellow in hybrids. The genetic ratios obtained suggest that a complex group of factors are present and that they must be in subtle balance. Furthermore, certain unknown metabolic factors are at play which are capable of producing changes within the organism. MUNSELL (45) has shown that the red fleshy portion of fresh watermelon tissue when fed to rats as a sole source of vitamin A produces a satisfactory unit gain over an eight-week period. This would lead to the belief that either certain yellow carotenoids must be present in the tissue of the watermelon and that the red pigment would seem to be superimposed on the yellow, as is the case in the tomato, or that certain other antiophthalmic-anti-infective substances are present. Both VON EULER and KARRER state that of all the naturally occurring carotenoids only the carotenes have a provitamin A effect. It seems likely, therefore, that such changes in color in the tomato due to temperature, or lack of such changes in the watermelon, as herein reported, are not the result of a single enzymatic factor but that they are the result of a subtle balance of conditions occurring in the actively metabolic, living cells.

#### Effect of ethylene upon the production of color in the tomato

Observations in regard to the physiological activity of ethylene go back to the early work on the effect of illuminating gas on plants. GIRARDIN (23) was the first worker to describe injury to street trees due to illuminating gas

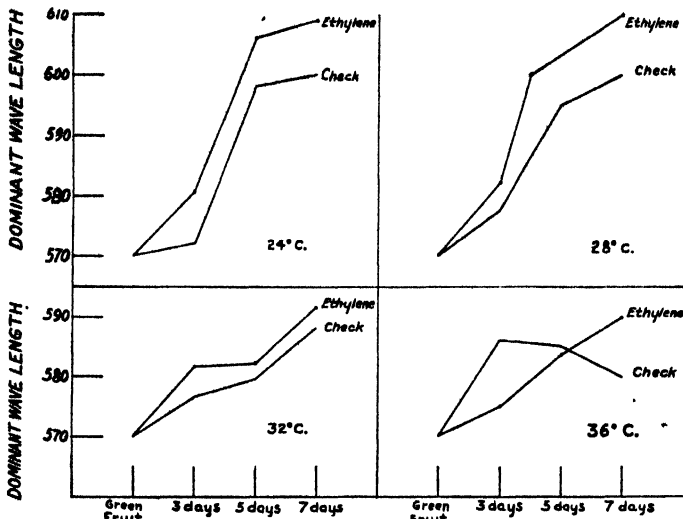


FIG. 5. Effect of ethylene at different temperature.

and KNY (31) was the first worker to conduct experimental work on the injurious nature of illuminating gas to maple and linden trees. As early as 1884 MOLISCH (42) observed that the linear growth of corn roots was retarded by low concentration of illuminating gas. In 1901 NELJUBOW (46) obtained a horizontal curvature of pea and bean seedlings with a concentration of but one part ethylene to one million parts of air. CROCKER and KNIGHT (15) determined that the injurious effect of illuminating gas on the flowering of carnations was due to the amount of ethylene which it contained. Later, MOLISCH (43), and CROCKER and KNIGHT (30) demonstrated that the

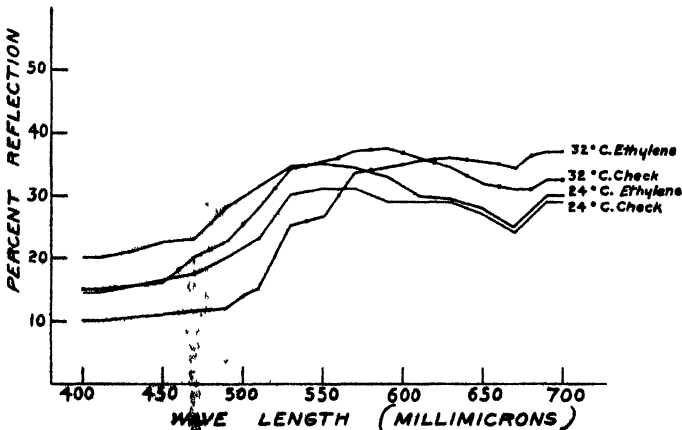


FIG. 6. Effect of ethylene (3 days, 24°, 32° C.).

physiological activity of tobacco smoke was due to the small amount of ethylene present.

SIEVERS and TRUE (53) observed the effects of the products of incomplete combustion of kerosene and other substances on the rate of coloring of citrus fruits. DENNY (16), trying empirically the substances in pure form which might cause this acceleration of color, was the first investigator to use ethylene for coloration of citrus fruits. By the use of ethylene, self-blanching varieties of celery were blanched by HARVEY (24) in six days with a concentration of ethylene of one part to one thousand at a temperature of 18° C. For the dark green varieties a longer time (ten to twelve days) was required. Positive results with ethylene on the ripening of tomatoes and bananas were also obtained by HARVEY (26) and successful commercial methods were worked out and established.

Recently ELMER (19) discovered that certain volatile substances from several varieties of apples caused an inhibition of growth of potato sprouts. GANE (22) was able to identify analytically the active volatile substance as ethylene, the amount produced being very small. NELSON and HARVEY (47), using the leaf epinasty method, were also able to show that Golden self-blanching celery produces in normal metabolism some gaseous or volatile compound and that the decomposition of chlorophyll is accomplished by some process similar to that by which celery is commonly blanched artificially by ethylene.

TABLE IV  
EFFECT OF ETHYLENE ON PRODUCTION OF COLOR IN TOMATO

	BRIGHTNESS		DOMINANT WAVE LENGTH		PURITY	
	ETHYLENE	CHECK	ETHYLENE	CHECK	ETHYLENE	CHECK
Vine ripened .....	.....	17.68	.....	605.0	.....	32.0
Green fruit .....	.....	30.45	.....	570.0	.....	40.0
24° C. 3 days .....	29.07	32.61	580.5	572.0	39.5	28.0
28° C. 3 " .....	26.00	32.27	582.0	577.5	43.0	28.5
32° C. 3 " .....	28.11	33.94	581.5	576.5	58.0	47.0
36° C. 3 " .....	38.37	21.60	575.0	586.0	40.5	23.5
24° C. 5 days .....	17.26	16.66	606.0	598.5	29.0	36.5
28° C. 5 " .....	14.57	16.72	600.0	595.0	36.0	30.5
32° C. 5 " .....	30.50	34.03	582.0	579.5	47.5	39.0
36° C. 5 " .....	34.78	28.45	583.5	585.0	45.0	44.0
24° C. 7 days .....	15.26	16.74	609.0	600.0	31.5	30.0
28° C. 7 " .....	14.71	17.91	610.0	600.0	32.0	35.0
32° C. 7 " .....	23.01	22.32	591.5	588.0	44.0	42.5
36° C. 7 " .....	20.43	26.02	590.0	580.0	40.5	32.0

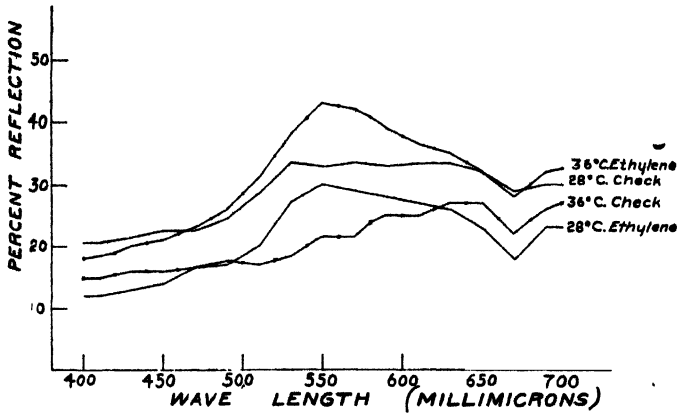


FIG. 7. Effect of ethylene (3 days, 28°, 36° C.).

The production of the red color of the tomato is due to the formation of lycopene within the actively metabolizing cells of the fruit. For this red color to be manifest the chlorophyll must first be decomposed. The data presented in table IV show that, under the influence of ethylene, the decomposition of chlorophyll is hastened and that the production of lycopene quickly follows, providing the temperature is suitable.

Bonny Best tomatoes were used in this work and received ethylene gas at the rate of one part per thousand on three successive days. The fact that ethylene-ripened fruits are redder than untreated ones in the same length of time is clearly demonstrated when dominant wave length is plotted against time, as shown in figure 5. However, it should be clearly stated that lycopene formation does not take place at temperatures of 32° or 36° C. whether

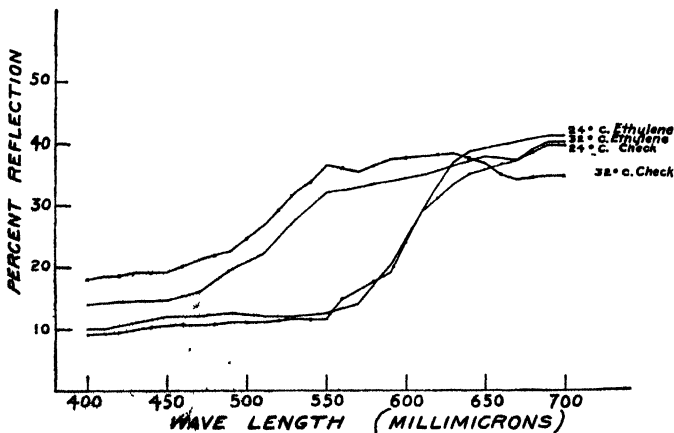


FIG. 8. Effect of ethylene (5 days, 24°, 32° C.).

treated with ethylene or not. Undoubtedly, some of the commercial failures to hasten the reddening of tomatoes by the use of ethylene are due to the fact that lycopene formation has such a narrow temperature range. Fruits ripened at 32° C. or above possess a dominant wave length of 575 to 591.5 m $\mu$  and a relatively high purity. Such fruits appear a bright yellow to the eye. When these yellow fruits are returned to a temperature of 20° C. to 28° C., lycopene formation occurs rapidly. However, such yellow fruits can be maintained in the yellow condition for from two to three weeks by storage at a temperature of 2° C. Evidently lycopene formation does not necessarily follow chlorophyll decomposition, and if lycopene is formed from colorless chlorophyll decomposition products, its synthesis involves a mechan-

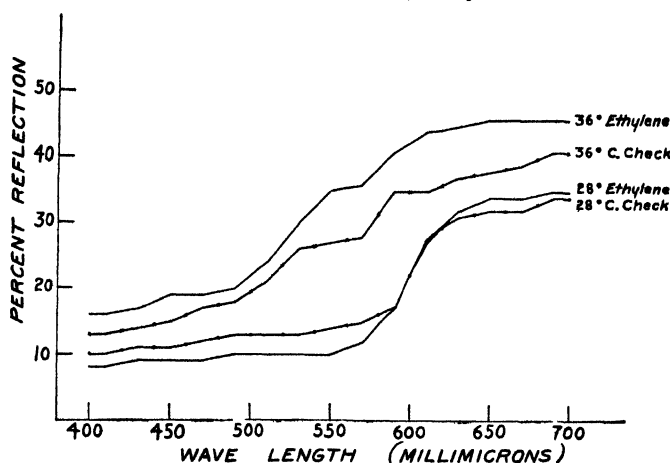


FIG. 9. Effect of ethylene (5 days, 28°, 36° C.).

ism different from the mechanism responsible for chlorophyll decomposition. The data in table IV (plotted in fig. 5) also show that ethylene hastens lycopene formation as well as chlorophyll decomposition. The ethylene-treated fruits possess a dominant wave length of 600 to 606 m $\mu$  at the end of a five-day-ripening period whereas the dominant wave length of those fruits ripened without ethylene varies from 595 to 598.5 m $\mu$  and appears more orange-red to the eye than those of the ethylene group (figs. 8, 9). Likewise, at the end of a seven-day period the ethylene-ripened fruits show a higher dominant wave length and thus would appear redder to the eye (figs. 10, 11).

Little is actually known in regard to the mode of action of ethylene in the living cell. A review of the literature shows that ethylene is exceedingly active and effects a great diversity of reactions. It is capable of markedly modifying the growth rate of meristematic tissue; of producing intumescences in many different plant tissues; of bringing about premature abscission of leaves; of producing specific epinastic response of petioles at a



minimum concentration of but one part in ten millions; of greatly increasing the oxidation rate in tissues exposed to it even in very low concentrations; of giving increase in soluble sugars and amino acids, at the expense of the insoluble carbohydrates, proteins, and fats; and of hastening the process of chlorophyll decomposition. Data presented above adds weight to the latter statement and, in addition, show that lycopene formation is materially hastened by ethylene, provided a suitable temperature is maintained.

### Effect of varying concentrations of oxygen upon production of color in tomato

In agreement with EULER (2) and KARRER (20), LUBIMENKO (36, 37) holds the view that the process of ripening in the tomato is an enzymatic

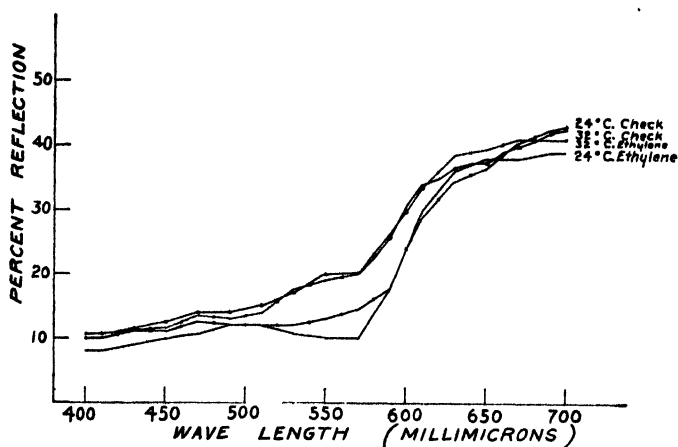


Fig. 10. Effect of ethylene (7 days, 24°, 32° C.).

one. However, he maintains that three separate processes take place in ripening. In the first period when the synthetic reaction is not crowded by oxidation, the accumulation of chlorophyll takes place. Later, oxidation processes predominate, whereby the chlorophyll together with the yellow components are destroyed and transformed into colorless unknown materials. His third postulate has to do with the color-producing activity of the plastid and the accumulation of a red carotenoid pigment within the cells. Whether or not these oxidation processes represent a part of what actually takes place in the cell remains to be seen. Preliminary work on ripening tomatoes in oxygen and other gases indicated that lycopene formation occurs only under conditions favorable for normal metabolism. Green tomatoes placed under pure carbon dioxide and nitrogen gases undergo no development of lycopene regardless of the temperature at which they are held. After five to seven days, such fruits undergo very serious internal breakdown, followed by rapid putrefaction. When the oxygen supply is

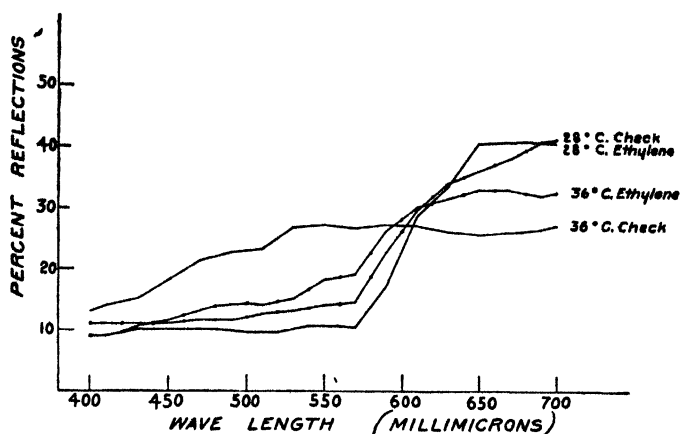


FIG. 11. Effect of ethylene (7 days, 28°, 36° C.).

inadequate, the formation of lycopene does not take place even under favorable temperature conditions. However, chlorophyll decomposition goes on uninterruptedly at low concentrations of oxygen, and at temperatures of 24° and 28° C., as well as at 32° and 36° C., yellow tomatoes are produced (figs. 12-16). Table V shows the dominant wave length of such yellow fruits to be 581.5, 578, 580.5, and 585 mμ at 24°, 28°, 32°, and 36° C. respectively.

Fruits ripened at low and high oxygen concentrations respectively were placed in the constant-temperature cases previously mentioned but within desiccators of approximately three and one-half liters capacity, four fruits being placed in each. Low oxygen concentrations were maintained by keeping the fruits in closed desiccators, which were ventilated with a stream of air for five minutes each twenty-four hours. High oxygen concentration was

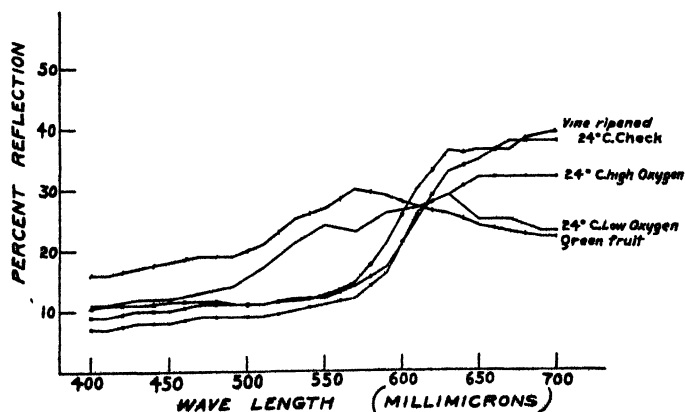


FIG. 12. Effect of oxygen concentration, 24° C.

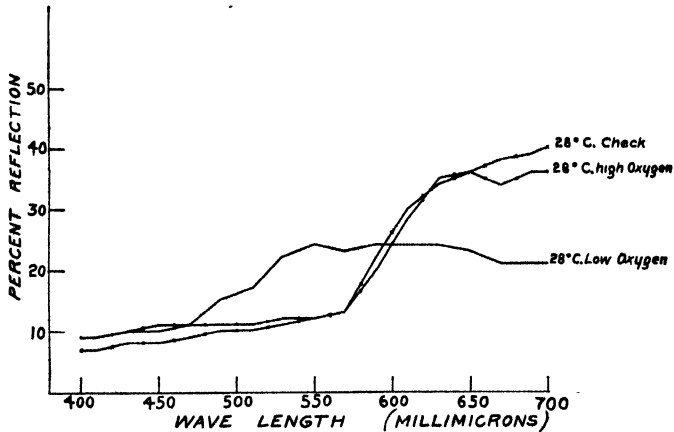


FIG. 13. Effect of oxygen concentration, 28° C.

maintained by slow, constant bubbling of a mixture of 50 per cent. air and 50 per cent. oxygen through the desiccators, the mixture being prepared in and liberated to the desiccators from individual gasometers.

Yellow fruits which were produced under low concentrations of oxygen reddened when returned to the air at 20° C. for four or five days, as also did the yellow fruits which were ripened at 32° and 36° C. in the high oxygen chambers. The close agreement of the dominant wave lengths of fruits ripened at 32° C. is remarkable, the fruits ripened in a low concentration of oxygen having a dominate wave length of 580.5 mμ, the fruits ripened at a high concentration of oxygen having a dominant wave length of 579.5 mμ, and the fruits ripened in air, not being confined to desiccators, having a dominant wave length of 582.0 mμ (fig. 14). The same is true of

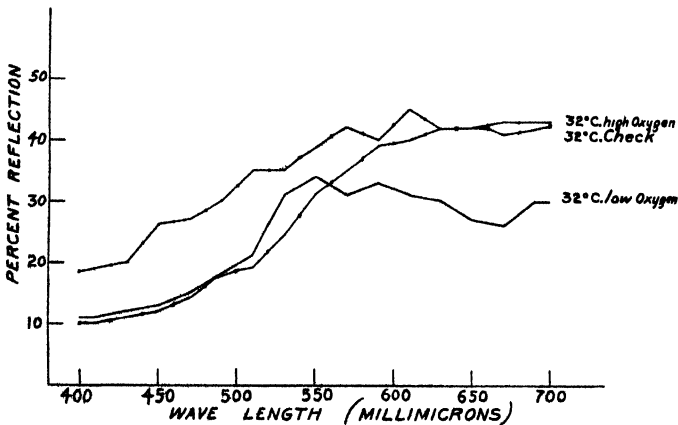


FIG. 14. Effect of oxygen concentration, 32° C.

TABLE V

EFFECT OF VARYING CONCENTRATION OF OXYGEN ON PRODUCTION OF COLOR IN TOMATO

	DOMINANT			BRIGHTNESS WAVE LENGTH			PURITY		
	Low O <sub>2</sub>	High O <sub>2</sub>	CHECK	Low O <sub>2</sub>	High O <sub>2</sub>	CHECK	Low O <sub>2</sub>	High O <sub>2</sub>	CHECK
Vine ripened .....	.....	.....	16.93	.....	.....	605.0	.....	.....	31.5
Green fruit .....	.....	.....	26.26	.....	.....	577.5	.....	.....	37.0
24° C. 7 da. ....	22.84	14.18	15.73	581.5	598.5	600.0	44.0	40.0	32.5
28° C. 7 da. ....	22.06	16.76	16.06	578.0	601.0	595.5	49.0	35.0	46.0
32° C. 7 da. ....	27.79	38.68	30.81	580.5	579.5	582.0	52.0	35.0	57.0
36° C. 7 da. ....	23.55	21.10	33.39	585.0	583.5	583.5	54.0	43.0	51.5

those fruits held at 36° C. having a dominant wave length of 585.0, 583.5, and 583.5 in low oxygen, high oxygen, and air respectively (fig. 15). This indicates that chlorophyll decomposition goes on at both low and high concentrations of oxygen but that the formation of lycopene is prevented when the oxygen supply is decreased, as in the case of fruit ripened under the partial anaerobic conditions of the experiment. That temperatures above 30° C. inhibit the production of lycopene is again apparent, which together with the before mentioned observation that suitable quantities of oxygen must be present for lycopene formation to take place indicates that beyond all reasonable doubt, the production of lycopene is a process which can take place only when the cells are actively metabolizing and that it seems to be more than a simple enzymatic change.

#### Effect of light upon the production of color in the tomato

The discovery by HARVEY (27) of the importance of light as a factor in the decomposition of the carotenoids present in butter and in the peel of

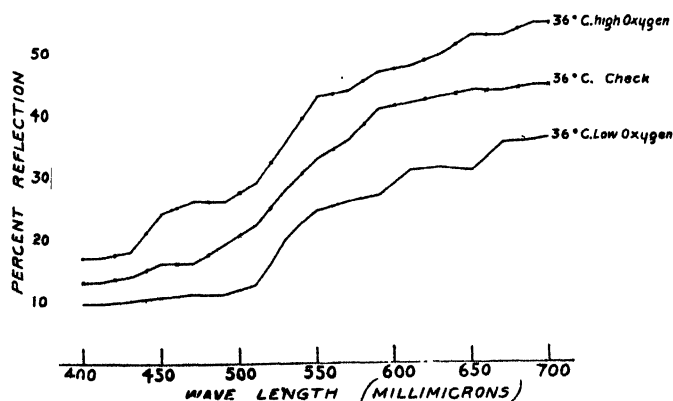


FIG. 15. Effect of oxygen concentration, 36° C.

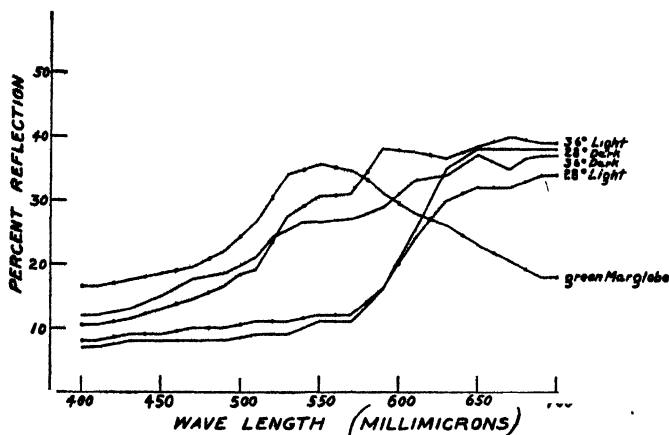


FIG. 16. Effect of light, 28°, 36° C.

orange fruits in the presence of benzaldehyde led to a series of experiments in which Marglobe tomatoes picked in the full-grown, green stage were ripened at temperatures of 24°, 28°, 32°, and 36° C. in total darkness and illuminated for twenty-four hours daily. The light source was a 1000-watt Mazda bulb giving an illumination of 1540 to 1620 foot-candles as measured by the Weston photronic cell, model 603. That chlorophyll decomposition is hastened by light is not denied but from the data presented in table VI lycopene formation is shown to proceed equally well in either light or darkness, provided the fruits are maintained in the presence of air and at a suitable temperature, namely, 20° to 28° C. (figs. 16, 17). Also, light may affect the yellow carotenoid constituents of the tomato, but the data indicate that temperature is the limiting factor in lycopene production. This also seems to hold true for the decomposition of other carotenoids. It was considered that light absorbed by chlorophyll and by carotenoid pigments might activate the decomposition of these pigments owing to the energy absorption,

TABLE VI  
EFFECT OF LIGHT ON PRODUCTION OF COLOR IN TOMATO

	BRIGHTNESS		DOMINANT WAVE LENGTH		PURITY	
	LIGHT	DARK	LIGHT	DARK	LIGHT	DARK
Vine ripened fruit .....	11.80	.....	609.0	.....	24.5	.....
Green fruit .....	31.21	.....	562.0	.....	40.0	.....
24° C. 9 da. ....	11.68	11.38	604.0	602.5	30.0	33.0
28° C. 9 da. ....	14.70	14.17	599.0	604.0	34.0	38.5
32° C. 9 da. ....	33.30	36.83	581.0	580.0	48.0	51.0
36° C. 9 da. ....	30.27	28.31	581.5	582.5	54.0	44.0

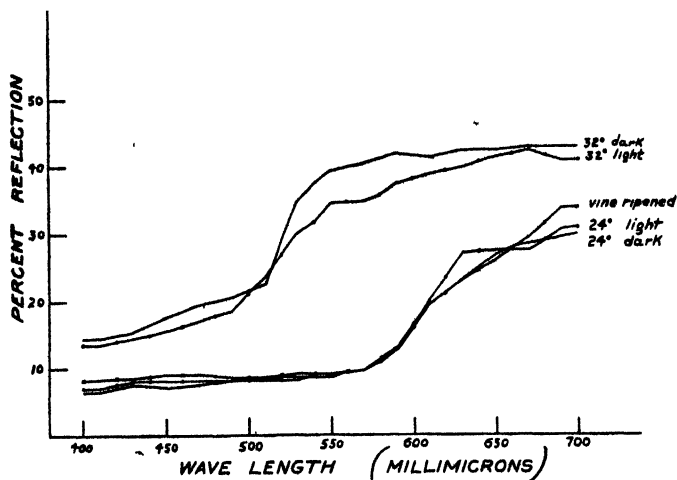


Fig. 17. Effect of light, 24°, 32° C.

but the data here presented indicate that this physical factor does not accelerate chlorophyll decomposition or the removal of carotenoids from the fruit in an appreciable amount. The data of HARVEY were obtained in the presence of benzaldehyde which may form active peroxides on light exposure of wave lengths which are absorbed by the carotenoids, and thus lead to their rapid decomposition. The tomato evidently does not produce such aldehydes as appear in the seeds of certain rosaceous plants. However, further explanation must await further progress in the explanation of certain internal changes which take place within the plasma complex of the living plastid.

### Summary

1. The optimum temperature for lycopene formation in the tomato was found to be 24° C. Lycopene is not formed at temperatures above 30° C. When tomatoes are ripened at 32° to 38° C., bright yellow fruits are produced. When such yellow fruits are returned to 20° to 24° C., lycopene is developed normally.

2. Chlorophyll decomposition in tomato fruits is prevented by a temperature of 40° C. or higher. Such fruits remain green and neither produce lycopene when returned to a temperature of 20° to 24° C. nor the yellow pigments when returned to 32° C.

3. In the case of the watermelon, a shift in temperature from 20° to 37° C. did not check the production of the red pigment, indicating that the same mechanism for lycopene formation does not exist in the watermelon as in the tomato. It seems likely that color changes in the tomato due to temperature, or lack of such changes in the watermelon, are not the result

of a single enzymatic factor but are the result of a subtle balance of conditions occurring in the actively metabolic cells.

4. Ethylene hastens lycopene formation as well as chlorophyll decomposition in the tomato, provided a suitable temperature is maintained.

5. Lycopene formation is prevented when the oxygen supply is decreased, chlorophyll decomposition going on uninterruptedly within the 24° to 36° C. temperature range.

6. Chlorophyll decomposition is hastened by light, but lycopene formation proceeds equally well in either light or darkness provided the fruits are maintained in the presence of air and at a suitable temperature.

The work here reported was performed in the Laboratory of Plant Physiology, University of Minnesota, and the writer wishes to acknowledge the assistance and encouragement of Professor R. B. HARVEY. Thanks are also due Professor R. A. GORTNER, University of Minnesota, for loan of the spectrophotometer, and to the Keuffel and Esser Co., Hoboken, New Jersey, for their helpful suggestions.

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# EFFECTS OF IONIZED AIR AND OZONE ON PLANTS

CLYDE HOMAN

(WITH FIVE FIGURES)

## Introduction

Beginning as far back as the latter part of the eighteenth century, numerous investigators have subjected plants to the influence of electrical fields of widely different potentials and frequencies, using various methods of application, often with the idea of obtaining some growth-stimulating effect. Adequate controls were generally not maintained, nor were the conditions used possible of duplication owing to lack of measurements of environmental factors. Although results were reported varying from greatly increased yields to disastrous effects, later extensive and more carefully controlled experiments (2, 6) tend to disprove any effect on growth because of potential gradient alone.

About 1914 it was suggested that such effects on growth, as were observed when using electrical discharges, were not due to the effect of a potential difference on physiological functions of the plant, but merely to secondary factors resulting from the electrical discharge, *e.g.*, formation of ions, ozone, active oxides of nitrogen, etc., (5, 10, 21). As far as can be found from the literature, there have been no experiments in which the mentioned factors have been isolated. There has been no work done in which these factors have been measured by any reliable method, either when attempts were made to isolate them, or under the conditions of a high tension discharge.

Workers who have used ultraviolet rays, x-rays, and radioactive materials, as well as electrical discharges, have employed agents that ionize the air and produce ozone to a greater or lesser extent; and to them a knowledge of the effects of these factors is of interest.

## Results of previous investigators

The first discussion of ionized air in this connection occurred in such papers as those of BRESLAUER (1) and JØRGENSEN (10). These papers refer to simple experiments with overhead wires, with the assumption that the air is more or less ionized by such discharges, but making no mention of measuring the ionization, if any, that may have occurred in the vicinity of the plants.

More recently experimental methods have been developed to determine whether ionized air has a direct influence upon plant growth. However, an analysis from the physicists' point of view of the previous experimental work with ionized air discloses a number of errors in method and interpretation sufficiently serious to invalidate most of the conclusions reached. Some of the most obvious of these errors may be pointed out.

Using polonium on copper foil as an ionizing agent for air, which was then passed over the plant material, MIDDLETON (16), WHIMSTER (29), VAN ASPEREN DE BOER (28) and PORTSMOUTH (19), obtained contradictory results. MIDDLETON found an increase in respiration varying from  $5.58 \pm 2.57$  to  $29.11 \pm 5.62$  per cent. PORTSMOUTH, attempting to duplicate MIDDLETON's results with barley seedlings, got no significant increase in one experiment, and only 1.5 per cent. in another. Again, WHIMSTER found an increase as high as  $85.7 \pm 7.1$  per cent. in respiration of *Pelargonium* leaves, while PORTSMOUTH with a similar experimental setup obtained no sign of the increase found by WHIMSTER. Likewise VAN ASPEREN DE BOER found no effect of ionized air on the respiration of fungi.

These men assumed that only alpha particles are emitted by polonium. However, RUSSELL and CHADWICK (20) found both a gamma and a beta radiation, the gamma being three times as strong when the polonium was deposited on copper as when deposited on aluminum. Therefore the results of the experiments given above may have been influenced by these gamma and beta radiations.

In these experiments, moreover, the number of ions per cc. of air was calculated from the saturation current and the volume of air between two plates placed in the position occupied by the plant material used. Of course the field would extend beyond the plates, and ions would be drawn from a considerable distance; but it is of much greater importance that the use of such high potentials as 947 volts across plates 2-5 cm. apart resulted in the electric field simply drawing the ions to the plates as fast as they were formed (the polonium being at a distance of only 4-5 cm.). What they really calculated was the rate of production of ions. The *number of ions per cc.* would not be measured by this procedure. The amount of ionization at a given moment would depend partly on the rate of recombination of ions, which would greatly lessen the number, as would also diffusion of ions and the movement of air. The voltage used was so high that no other evidence is needed that the ions were drawn to the plates as fast as formed, the mobilities of the ions being about 90 cm. per second.

SPOEHR (23) drew air, supposedly ionized by sunlight, from outdoors over wheat seedlings and onions, and measured the  $\text{CO}_2$  output during the day and night. He found that when the air was not de-ionized by passing through an electric field, the ratio of  $\text{CO}_2$  output per hour by day to that by night was 1.15 for onions and 1.042 and 1.091 in two studies of wheat seedlings. In two experiments, in which he de-ionized the air drawn in before passing it over the plants, the ratios were 1.010 and 1.015. This would indicate that air exposed to daylight with its slightly higher degree of ionization caused slightly increased respiration, provided no other changes had also been brought about. It would seem, however, that drawing the air

through 45 feet of glass tubing in a thermostat would allow sufficient time for considerable recombination to take effect, possibly nullifying the higher ionization due to the sun's rays. There was no measurement of the degree of ionization of the air, reliance being put on the work of other investigators, which shows, in general, that there is a higher ionization of the air in sunlight than in darkness. There has evidently been no confirmation of this work.

HENRICI (7, 8) noted in the course of her experiments that the assimilation of alpine plants was much more active before a thunderstorm in spite of the low intensity of the light. She ionized the air artificially by the use of thorium oxide, and protected the plants by aluminum screens from any alpha radiation. She found that for lowland plants ionization favors assimilation in weak light, has no effect in medium light, and retards it in strong light; for alpine plants ionization increases assimilation in weak and medium light, and has no effect in strong light. She claims that in suitable conditions of light, ionized air increases photosynthesis; in some cases she got as high a ratio as 3-4:1. Her results, however, were not based on the averages of a sufficiently large number of experiments for calculating the probable error; hence it is not possible to estimate their value. Calcium hydroxide solution in Pettenkoffer tubes was used for absorption of  $\text{CO}_2$ , and it does not seem that the small differences in  $\text{CO}_2$  found could have been very accurately measured. She has not continued with this work as far as can be determined from the literature, and there has been no confirmation from other sources. No measurements as to degree of ionization produced were made. Methods used to control temperature, etc., were not clearly described. Also, thorium oxide is a very weak ionizing agent, and aluminum sheets would not stop gamma rays.

LIPPERHEIDE (14), using sparks as a source of ionized air, presented the results and conclusions of a number of experiments mainly on the supposed effect of ionized air under the conditions in which he produced them. The conditions of his experiments are briefly as follows: Potted bean plants, *Phaseolus vulgaris*, were grown in garden soil in cases with glass tops. The condition of the air in the treated case was modified by blowing air through two metal combs placed opposite each other. Sparks were passed between the points by connection with an induction coil and other necessary electrical apparatus. Though he does not state the current or sparking distance used, the coil had a maximum capacity of 18 amp. and would give a spark 20 cm. long.

Only a relative idea of the degree of ionization in the treated air as compared to the control was obtained. This was determined by the time it took to discharge a metalfoil-leaf electrometer sufficiently to cause the leaf to move five divisions on the scale. Roughly, the degree of ionization would

be inversely proportional to the time elapsed. The peculiar thing about his figures is that they show great variability in the time required for this degree of discharge of the electrometer. In the control case the time varied from 1 minute and 40 seconds, to 4 minutes, most of the readings being about 4 minutes. In the treated air the variation was from 7 to 50 seconds, mostly around 10 seconds. Thus, the average degree of ionization of the treated air was roughly 24 times that of the control. This gives an ionization of only 24,000 per cc., if the ionization of the control air is assumed to be 1000 per cc. This is much lower than has been obtained by the writer and others referred to, and indicates that the sparks used were not of great number and the field strength comparatively weak. This probably accounts for his being able to grow plants under the conditions employed, for with a sufficiently weak field and sparks the quantity of ozone would be kept below toxic concentration limits. However, he must have been near the border line, for he states: "Die Schädigung durch Ozon war bei manchen Versuchen so gross, dass oft ganze Versuche abgebrochen werden mussten." His results show, as summarized also by KOERNICKE (13), that 35 control plants had a leaf surface of 741.37 sq. cm. while 35 treated plants had 1,431.32 sq. cm.; and 31 control plants had a dry weight of 19.057 gm. while 31 treated plants had a dry weight of 33.655 gm. Control plants transpired, during five days, 1299 gm. of water, and the treated plants 1554 gm. Also, during three weeks, when the plants were grown in a nutrient solution, the intake of salts was 7 mg. higher per treated plant than for the controls. Other experiments showed that when the plants were placed in a cellar, a three-peaked curve of leaf motion of small amplitude was obtained, which became a normal two-peaked one of larger amplitude again after "ionization." With stronger ionization, stronger movements were obtained, and with strong ionization the leaves rose. He claims that plants in ionized air remained green in the dark for a longer period; and that when grown in "ionized" air they were darker green and often had a fourth leaflet. This latter observation was emphasized and photographs recorded. This emphasis seems unreasonable. It so happened in the writer's work that this fourth leaflet appeared frequently, but more often in the untreated plants than in the treated.

The above results alone are interesting, but the following criticisms make them untenable:

(1) At all times LIPPERHEIDE used both ionized air and ozone, with possibly small amounts of nitrogen oxides, the amount of  $O_3$  produced depending on humidity, potential used, etc. Up to the time his work was done there had been nothing to show that  $O_3$  in small dosages might not be beneficial. Therefore, assuming all other conditions constant, one would not know to which to attribute the effect. One could possibly assume that when the concentration of  $O_3$  passed a certain point the plants were harmed. This

evidently often occurred. Also, he gives no data on point distances, current, or potential used.

(2) Evidently there was no method of controlling air flow. It seems that all that was done to the control case was to open the door while he was blowing the air past the discharge into the treated chamber, "Dadurch wurde es vermieden, dass den Versuchspflanzen mehr frische Luft zugeführt als den Kontrollen."

(3) How light was controlled is not discussed. It seems it would be very difficult to place two cases in a greenhouse so that the light penetrating the cases was at all times equal.

(4) Where soil was used, no mention is made of how soil moisture was controlled.

(5) Definite mention, however, is made that the humidity varied considerably at times when the readings given were made, there being variations of 25 to 30 per cent. Also some differences are given for temperature. The blower connected with the treated case was evidently only in operation when a discharge was going on between the points. During the remainder of the time, the door of the control case was opened an amount supposed to approximately equalize the effect of the ventilator of the treated case. There is nothing in the description of the construction and setup of the cases which would show that a significant difference in humidity might not have existed most of the time. The writer's work with the same plants indicates that *Phaseolus vulgaris* is particularly susceptible in its growth and size of leaves to moisture conditions.

(6) The work may be criticized because of the small number of plants for which data are given; also, it is not clear on what basis the plants thought to be injured by ozone were eliminated.

(7) From the diagram shown in his article, it appears that the ultra-violet light from the sparks might reach the plants, perhaps having some effect.

In all other experiments listed in the bibliography, no measurements were made, as for instance in the numerous experiments of NEHRU (17) in which a variety of sources of supposed electrical energizing were used and seemed in almost all instances to produce stimulating effects.

Lack of controlled conditions as to light, temperature, air flow, etc., are general, as is also the use of a limited number of plants, or the taking of readings of respiration over very short periods, or other similar procedures which would tend to emphasize idiosyncrasies of individuals or of small groups, or differences due to time of day and other local factors. An example of this is the case in which NEHRU (17) after uprooting 5 plants, treated them with an electric spark and used only one plant for a control.



Then there exists in some cases questionable accuracy of measurements by absorption or colorimetric methods of the small amounts of  $\text{CO}_2$  involved in small differences in respiration.

### Available information on effects of ozone on plants

The earliest mention of studies of the effects of ozone on plants was found in a report by HABER (5) of the Kaiser Wilhelm Institute who presented his results at a special meeting. This work has remained unpublished, but the following quotation is taken from the above report:

"He gave the results of his investigations with others of the assimilation of a leaf of cherry laurel in air filled with carbonic acid. It was found that the electric field, either continuous or alternating, produced no change in assimilation unless a glow discharge was obtained. The reaction produced by the glow discharge injured assimilation. The admixture of ozone or oxide of nitrogen with air had the same effect."

The information that this work remained unpublished is taken from STERN (24).

KNIGHT and PRIESTLY (11) passed air through a jar in which an electrical discharge was taking place and thence into a jar containing seedlings. They found the chlorophyll in the upper portions of the seedlings had been bleached. They made no measurements of the products of the discharge but concluded there was no effect on respiration. The bleaching, as will be shown later, was, no doubt, due to a fairly high concentration of ozone.

LIPPERHEIDE (14), in a short paragraph on the influence of ozone on leaf movements of *Phaseolus*, states that the leaves go into the "sleep position" when subjected to a moderate amount of ozone. He finds that the leaves are very sensitive to ozone. If ozone was present for over five minutes, the plants were injured, and after two days were speckled brown, and parts were dead. He adds that ultraviolet rays also cause a downward movement of the leaves and attributes this to ozone arising from the action of ultraviolet radiations on air. No analysis of the gas used was made.

According to authoritative opinion (9), ozone in sufficient concentrations to kill bacteria will kill animals. Apparently, beneficial effects of ozone are due to its odor, which gives relief to the olfactory sense from the tedium resulting from constant inhalation of air at constant humidity and temperature. Also, beneficial effects in diseases of the mucous membranes, if any, are the result of blistering action which may cause increased blood supply.

TAKAMIYA (25), in repeated experiments, has shown that ergosterol exposed to ozone in proper concentration gives a substance having the action of vitamin D, as it also does in ultraviolet light. Thus there might be some beneficial reaction with plant sterols, although work reported in this paper does not indicate it.

Ozone is not found to any extent in lower altitudes due to its decomposition by moisture. In the upper stratosphere, measurements have shown (15) that there is an equivalent of a layer about 4.2 cm. thick. MELLOR (15) emphasizes the importance of this by quoting B. MOORE as follows:

"As the light from the sun first strikes the earth's atmosphere it is full of ultra-violet light and violet light. Such light rays as these, if they reached the earth at the present moment, would annihilate us; they would simply mean death to the world below. The first thing that happens to prevent this is that ozone is developed, and by absorption this ultra-violet light is shut out."

In regard to the possible production of ozone by plants, URSPRUNG and GOEKEL (27) obtained an effect on a photographic plate placed in a closed chamber with plants. As their electrometer showed no change, they concluded that the effect on the plate was due to ozone or other gaseous products and not to ultraviolet radiations which would ionize the air. Results that seem to confirm this idea were given by COUPIN (3). A concentrated solution of KI in litmus paper was placed in a chamber with plants and also in a control chamber, and exposure made to diffuse light. The litmus turned blue only in the plant chamber. Evidently some oxidizing agent, perhaps ozone, was formed by the plants which liberated KOH.

### Plan of experiments

From the summary just presented, the writer concludes that little can be definitely said, except that  $O_3$  at certain high concentrations was undoubtedly toxic. The possibility remained that ionization alone might have been stimulating, or that ozone in proper concentration might be beneficial to growth. It was, therefore, decided to carry out the following plan:

- (1) Determine a method of growing a number of plants at a time under exactly controlled conditions so that it would be possible to change only the gaseous environment of the plants.

- (2) Find the best method of producing a high concentration of gaseous ions uncontaminated by ozone.

- (3) Devise a method of producing ozone in various desired concentrations without nitric acid anhydride ( $N_2O_5$ ) being present in any detectable amount.

- (4) Grow plants (*Phaseolus vulgaris*) for a sufficient length of time (seedling to blossoming) so that any effect on growth would become definitely evident.

- (5) Grow plants in different concentrations of ozone, to determine what concentration is toxic, the specific effects produced, and whether different small concentrations applied either constantly or intermittently are stimulating.

## Apparatus and methods

### GROWTH CHAMBERS

The plants were grown in experimental cases of the design and dimensions illustrated in figures 1 and 2. The top is set in a trough completely surrounding the sand pan and may be lifted off. This trough is filled with water or other suitable liquid, which acts as an air seal. The glass sides leave the plants visible, while the easy removal of the glass case from over the plants makes it convenient to get at them. To assure complete drainage, the bottom of the sand pan was slightly funnelled.

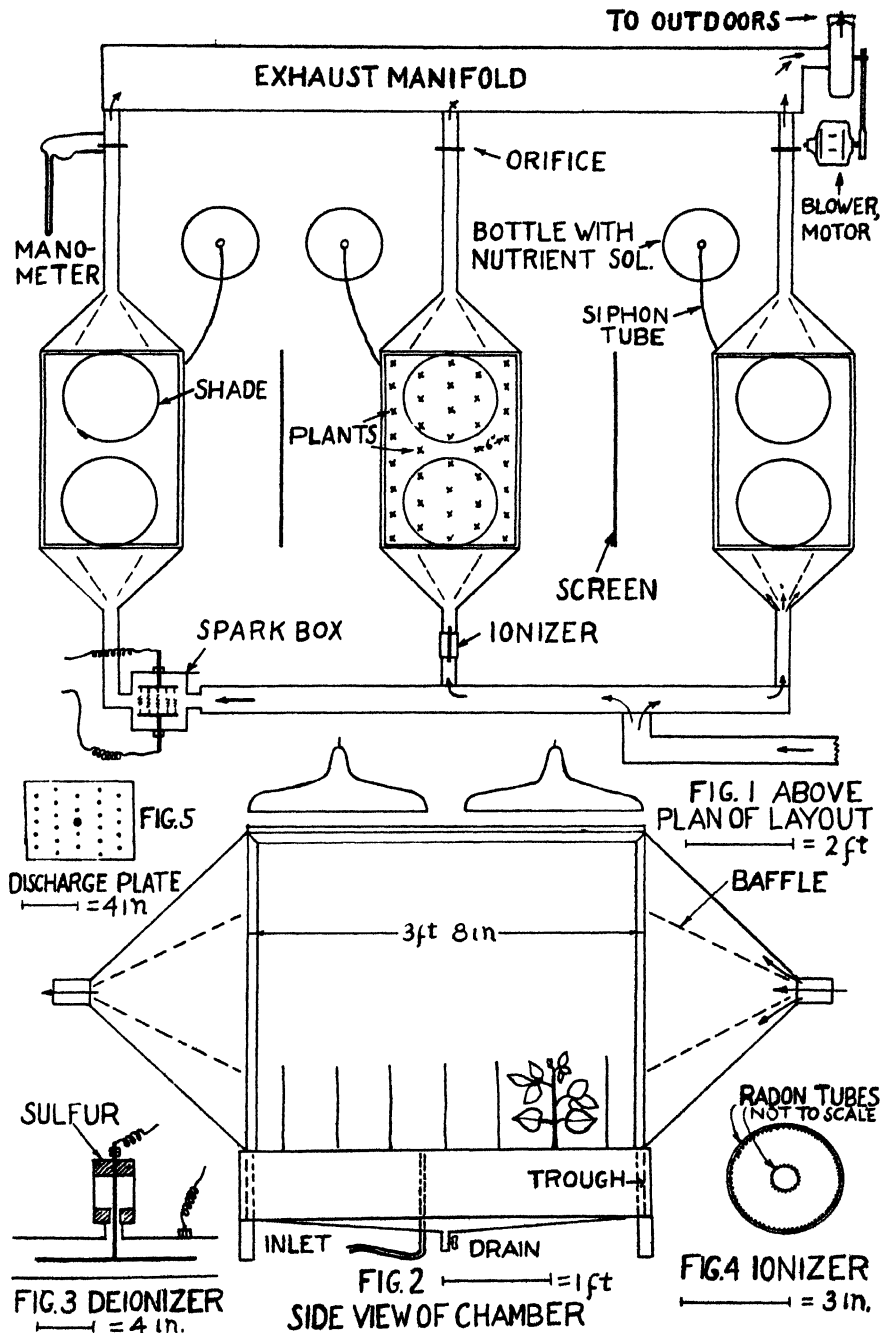
Baffles are soldered into the funnel ends so that air entering and leaving the case is spread out evenly through the interior and does not pass only through the center region. The baffles and funnels are painted dull black to avoid reflection of light. Around the top of the case is fastened a band so that the top glass may be covered with a layer of water.

Three cases were placed in parallel between a common outlet and inlet. One case served as a control for the other two, thus reducing by half the time which would be needed if only two cases were used. Air was drawn through the cases by an exhaust centrifugal blower. Lighting was accomplished by placing incandescent bulbs with reflectors over the cases. The gaseous products were introduced at the inlets.

### CONTROL OF AIR FLOW

In order to be able to maintain equal air conditions in the cases, it was imperative that the air flow be accurately measured and regulated. The common exhaust manifold was 15 inches in diameter so that there would be no drop in pressure with distance from the outlet.

Measuring and regulating air flow was accomplished by the method of differential pressure across an orifice plate placed in the line. The principle is based on BERNOULLI's theorem (12). If a pipe of perfectly true bore is used, and an orifice plate inserted so that a perfectly round hole is accurately centered, and taps then taken at proper distances, the flow through that pipe may be known with an accuracy of 0.5 per cent. Tables have been worked out (22), so that, knowing the fluid and its density, the inside pipe diameter, the ratio of the orifice to diameter of the pipe, and the differential pressure across the orifice, the flow may be found. Correction factors, (also obtained from tables) are introduced for temperature, pressure, and humidity. The tables used here were kindly furnished by the Republic Flow Meters Company of Chicago, Illinois. Of course, the accuracy of the determination of the flow depends entirely on the accuracy with which the diameter of pipe and orifice, and the differential pressure reading are obtained. Seamless steel tubing was used which varied only 1 to 2 thousandths of an inch, and sufficient length was used in front of the orifice so that any turbu-



Figs. 1-5. Plan of layout and chambers, with details of deionizer, ionizer, and discharge plate.

lence in the flow would be smoothed out. The flange, fitting over the pipes and holding the orifice plates, and the orifice plates themselves, fitted together with the orifice exactly centered. The size of the orifice and the speed of the blower were adjusted by trial so that the flow desired could be obtained with a differential pressure of 70–100 mm. of water, which differences could be read within a fraction of 1 per cent. Even greater accuracy than 0.5 per cent. could be obtained when simply balancing the flow through two or more identical pipes and orifices, and in reading the differential pressure for accurate adjustment. In this work the limit of error was not over 1 per cent. The greatest errors come from inaccuracies in knowing the diameters of the pipe and orifice, which are perpetuated as the square, while those in reading the differential pressure vary only as the square root.

The blower used was of the ordinary centrifugal fan type (Buffalo Forge Co. no. 1) and was driven by a synchronous motor to avoid changes in flow resulting from fluctuations in line voltage, which was found to vary as much as 10 per cent. when an induction motor was used. As the pressure on the exhaust side of all three orifices was the same, the flow was balanced through the cases by simply adjusting the inlets so that the differential pressure across the orifices were identical. Since the sizes of the three cases and pipes leading in and out of them were all the same, very little adjustment was necessary. It was found that a flow of about 30 cu. ft./min. was suitable, as this changed the air about once a minute in the cases, caused only very trifling movement of the leaves, and when ionized air was being used, was sufficiently rapid so that too large a degree of recombination did not take place. Since air entered the cases from a common manifold and passed through rather rapidly, it was not to be expected that the humidity would differ in the different cases. Measurements of the relative humidity in the three cases showed that there was no detectable variation.

#### CONTROL OF LIGHT AND TEMPERATURE

Over each case, at a distance of two inches from the glass, were suspended two 1000-watt bulbs with reflectors. These bulbs were tested for equality of illumination. The glass of the tops was  $\frac{1}{8}$  in. thick, the sides  $\frac{1}{8}$  in. and of a special high-grade crystal type of glass which was uniform in light transmission and thickness. The top glasses were covered with  $\frac{3}{8}$  in. of distilled water, maintained at constant level by an overflow and drip source of replenishment. As dust settled to a slight extent in spite of the fact that the experiment was carried on in an air-quiet basement room and that the reflectors largely covered the tops, the glasses were cleaned daily. Without the water on top, and with an air flow of 30 cu. ft./min., the temperature of air leaving the chamber was 2° or 3° C. higher than that entering; while with the water screen, it was only a fraction of a degree higher. The temperature

in all three cases was equal. But the water was used throughout, not particularly to lower the actual temperature of the air in the cases but to eliminate a large proportion of the infrared rays and so to give a better balanced spectrum and also to prevent internal heating of the leaves. It was found that the plants used showed no curvatures in any direction and grew to uniform heights. There was, therefore, little or no error introduced due to one set of plants growing nearer to the lights, which might tend to exaggerate the differences in growth. Light screens were placed opposite the glass sides of the cases to prevent light from one case being reflected into another.

#### CONTROL OF SOIL CONDITIONS

The plants were grown in pure quartz sand, eight inches deep. Constant soil moisture conditions were obtained by flooding once a day with nutrient solution and draining immediately after. This was accomplished by placing a five-gallon bottle of nutrient solution somewhat above the pans and allowing the solution to siphon to the surface of the sand through a  $\frac{1}{2}$  in. pipe from the bottom where it poured into a  $1\frac{1}{2}$  in. angle iron with holes bored along its sides. In this way, the liquid flowed fairly uniformly throughout the length of the pan into the sand without disturbing it. When the sand was immersed about  $\frac{1}{4}$  in. in the liquid, the inflow was shut off and the drain opened. As the sand in all these pans was of the same particle size and of the same packing tendency, the amount of solution held should have been practically identical in all three cases. That this was true was shown by measurements of the amount entering, and of the excess that could be drained. Thus soil moisture conditions were constant at the start and would be changed only by differences in degree of transpiration and evaporation during the twenty-four-hour period between waterings. However, there was ample moisture in the sand to last several days and tests showed that it took practically the same amount of water to saturate the sand in each of the three pans after twenty-four hours of transpiration and evaporation. Fresh air was also sucked into the sand when the liquid, filling the intergranular space, was drained.

The nutrient solution used was as follows:

A.	50 gm.	MgSO <sub>4</sub>	in 500 cc.	H <sub>2</sub> O
B.	50 "	KH <sub>2</sub> PO <sub>4</sub>	" "	" "
C.	50 "	Ca(NO <sub>3</sub> ) <sub>2</sub>	} " "	" "
	50 "	CaCl <sub>2</sub>		
D.	4 "	FeCl <sub>3</sub> · 6H <sub>2</sub> O	" 300 "	" "

Add 10 cc. of solutions A, B, and C, and 10 drops of D to four liters of distilled water.

## OBTAINING PLANTS OF SIMILAR SIZE AND VIGOR

About 1000 *Phaseolus vulgaris* seeds of uniform size were selected each time and planted in quartz sand. They were found to germinate uniformly and from this number of plants it was not difficult to obtain 99 plants of almost identical appearance at an early stage in the development of the primary leaves (about ten days after planting). Thirty-three were then planted in each pan (fig. 1), six inches apart and four inches from the sides, in the case of the two rows nearest the glass. At this distance there was little crowding and the plants were not interfered with nor shaded by one another. The plants grew very uniformly and no differences could be noted between the outside and inside rows.

## PRODUCTION AND MEASUREMENT OF IONIZED AIR

Three sources of ionization of the air drawn into the cases were considered: spark discharges, radioactive compounds, and x-rays. It was at first thought that spark discharges would serve best for a constant source of quantity production of ions and since LIPPERHEIDE used this method, it was tried first. A spark discharge box (fig. 5) was built. It consisted of two plates each having twenty-five points spaced 1 in. apart. These were capable of being adjusted so that the points of the opposite plates could be placed at any distance from one another up to 8 in. The two plates were connected to the terminals of the secondary of a transformer that could be regulated up to 100,000 volts. When the current was turned on, a lively display of sparks played between the points. Large Leyden jars (3000 m.m.f. capacity) were placed in parallel with the plates to prevent arching. In this way blue-white, crackling, thick, hot sparks were obtained, which type forms the least ozone. Air was then drawn between the points through the sparks into the plant chambers.

Another method of producing spark discharges was to use a Tesla coil discharge (frequency 4,500,000 cycles per second) connecting the two terminals of the secondary connected to the plates. This gave thinner purplish sparks that became a spray of fine thin sparks spreading between opposite points. This changed almost to the appearance of a glow discharge filling the space between the plates when the points were moved farther apart (6 to 8 in.). The thin sparks produced by the Tesla coil when the points were drawn 4 to 6 in. apart spread out from the points so that practically all the air between the points had a visible discharge in it.

In order to measure the number of ions per cc. in the air entering and leaving the plant chambers it was necessary to build a device which could be inserted in the air line and by which all of the ions could be discharged. As these were passing in at a constant rate, a constant current would result

electrometer was used (capacity 93 e.s.u.) because, by inserting different capacities in parallel, it could be made flexible in use over the range of currents to be measured and because a galvanometer that was sufficiently sensitive for some of the small currents to be measured was not available.

The device through which the ionized air passed into the chamber is shown in figure 3. It consisted of a brass tube of the same size as the pipe entering the chamber. A brass rod was suspended longitudinally down the center of the tube from its middle by another rod passing up through two cakes of sulphur held by a glass tube with its lower end drawn in but not touching the brass rod, so that the surface of the sulphur would not become contaminated from dust, moisture, etc., in the air passing through the tube. The use of sulphur was found necessary. At first, glass alone was used, and there was a considerable leakage over 12 inches of glass tubing from which the rod was suspended at the top, due to surface moisture. The outside tube, which was completely insulated from connecting pipes, was charged up to the proper voltage by connection to one pole of a battery, the other of which was grounded.

A lead was taken off the suspended rod to one quadrant. Because of the strong electric waves given off by the spark discharges, which would greatly affect the electrometer, it was completely shielded in a galvanized iron box, the beam of light for reading passing through a screen. The lead from the suspended rod and the part projecting up above the brass tube was shielded by metal tubing. It was not found necessary to place screens across the ends of the brass tube because the suspended rod, placed only within 2 inches of each end, was found to be so completely shielded as not to affect the electrometer. Then, by first placing the quadrant with suspended rod and lead at ground potential and with the outer tube charged, all ions of similar charge were driven to the brass rod where they gradually charged the quadrant up to the potential of the outer tube. The rate at which the quadrant charged up, and thus the rate of turning of the needle as measured on the scale, depended on the number of ions available to carry the current across from the outside tubes to the rod, and also on the capacity of the quadrant, lead, and rod. Knowing the capacity of the system (found by the usual method of calibrating with a known capacity), the charge of a single ion, the number of centimeters of scale corresponding to a change of 1 volt in the potential of the quadrant, and the rate of flow of air in cubic feet per minute, the number of ions per cc. may be calculated as follows:

$$2 \times \frac{(C_e + C_c) (V/cm.) \times (\text{number of cm.})}{t} \times \frac{28.6 \text{ cu. ft. per min.}}{60} = \text{no. of ions per cc.}$$

$$(4.77 \times 10^{-10} \text{ e.s.u.}) (30.48 \text{ cm.})^3 \times \frac{28.6 \text{ cu. ft. per min.}}{60}$$



**Explanation of symbols:**

Ce = capacity of electrometer and leads (93 e.s.u.)

Cc = capacity of air condenser inserted in parallel

V/cm. = number of volts to which 1 cm. corresponds on scale placed at one meter = 1.2

Number of cm. = number of cm. taken for reading

t = time in seconds for the needle to move the above number of cm.

$4.77 \times 10^{-10}$  e.s.u. = one elementary unit of charge, *i.e.*, charge on one ion

(30.48)<sup>3</sup> = conversion factor for changing cubic feet to cubic centimeters

28.6 cu. ft. per min. = rate of flow of air per minute, divided by 60 to change to seconds

2 = factor for total number of ions as only one-half of the ions (as measurements showed there were equal numbers of + and - ions) are driven to the quadrant of the electrometer, the other half being neutralized by the charge of the tube.

It was found that charging the outside tube to 135 volts was sufficient to take out all of the ions at the rate of air flow used. Further increase in voltage gave no greater rate of movement of the needle. In fact, 90 volts gave, within a small percentage, the same reading as 135 volts.

In calibrating the electrometer several radon tubes were used as a constant source of ionization. These were suspended in the brass tube. It was found that the needle swung extremely rapidly and, in order to get the motion down so that readings could be made accurately, almost all of the tubes had to be removed. It was subsequently found that by placing the radon tubes at the entrance to the plant chamber (air flowing at the time) and the ionization chamber at the outlet, a higher reading was obtained than if the air had passed through sparks. In order to see how many ions per cc. could be produced with a flow of 28.6 cu. ft./min., a glass tube was coated on the inside with several hundred old radon tubes and another smaller glass tube suspended longitudinally in the larger tube, it being coated on the outside with radon tubes (fig. 4).

It was thus found that 1,100,000,000 ions per cc. could be produced at the near end with a flow of 28.6 cu. ft./min. That this measurement was correct was shown by placing three feet of pipe between the ionization chamber and the radon tubes. No current could be detected by the electrometer unless air was flowing through the pipe, showing that what was being measured was actually the number of ions per cc. and not the rate of production of ions by rays of some sort. At this point adding additional radon tubes did not increase the ionization perceptibly.

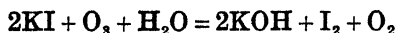
At the outlet of the chamber, owing to recombination of ions while passing through, the reading dropped so that only 21,000,000 ions per cc. were measured, but this is still a large number.

It was found that the Tesla coil discharge would give only about one-fifth of this number, and the maximum white spark discharge obtainable gave only about one-twelfth as many. This latter degree of ionization was obtained at a point distance of about 4.5 cm., when there was probably the greatest density of sparks and greatest potential drop per centimeter. Practically no ionization took place unless there was a spark or corona discharge. Sparking, and not the field alone, is evidently necessary to get a large degree of ionization by collision. This would indicate that in experiments with overhead wires little ionization takes place. The ionization readings remained constant and the maximum degree of ionization was used throughout the experiments using ionized air. Strangely enough, a spark discharge used as an auxiliary source with the radon tubes, decreased rather than increased the readings.

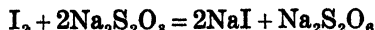
Owing to the fact that elaborate equipment would be needed to employ x-rays and eliminate errors and danger from their use, and that the tubes would not be constant sources, and could not be run for long periods, they were not tried.

#### MEASUREMENT AND PRODUCTION OF OZONE

The air from the plant chamber was drawn through two Fisher wash bottles containing KOH solution and through two containing neutral KI solution at a constant known rate. This rate was determined by calibration of a capillary flow-meter and kept constant by regulation of the differential pressure. It was found, in calibrating, that if the differential pressure was set at a certain point, the time of flow of a certain volume of air could be predicted within a fraction of 1 per cent. The rate used was about one-half liter per minute. The iodine liberated according to the equation



was titrated in acid solution according to the method described by TREADWELL and HALL (26).



or 2 moles of  $\text{Na}_2\text{S}_2\text{O}_3 = 1$  mole  $\text{O}_3$ . Thus knowing the volume of gas drawn through the absorption bottles and the number of cc. of 0.01 molar  $\text{Na}_2\text{S}_2\text{O}_3$  solution used, the number of parts of  $\text{O}_3$  is easily determined.

$$\frac{\frac{1}{2} \text{ number cc. of 0.01 M } \text{Na}_2\text{S}_2\text{O}_3 \times \frac{22,400}{1000 \times 100}}{(\text{Number of minutes of absorption}) (\text{cc. per min.})} = \frac{\text{Parts of ozone per one}}{\text{part of air.}}$$

(Corrected for standard conditions)

To produce  $O_3$ , and to be able to regulate the amount, a Siemens type of ozonizer was built (diameter 4 in.). Regulation of the concentration of ozone could be accomplished by changing the area of the outer layer of foil, by changing the potential, by changing the cross sectional open areas between the glass tubes or the amount of open area of the inside tube, thus causing more or less air to flow relatively between the tubes where it was ozonized by the strong alternating electric field. Twenty to thirty thousand volts could be used without sparking. The air, after being ozonized, was passed through the deionizer.

It was soon found that only small concentrations of  $O_3$  were necessary and at none of the concentrations used was any  $N_2O_5$  detectable by passing large volumes of the air through KOH and testing the resultant solution by several of the most sensitive qualitative tests for  $NO_3$ . All lower oxides that may be formed are immediately oxidized to  $N_2O_5$  by  $O_3$  (4).

### Experimentation

#### EXPERIMENTS 1 AND 2

Thirty-three seedlings were transplanted into each chamber. The primary leaves were about one inch across. Lighting was for 16 hours daily. Ionization was at a maximum rate and was continuous, using the radon tube device. The sand was saturated and drained every twenty-four hours.

The plants grew rapidly and bloomed nineteen days after transplanting, and twenty-nine days after planting of the dry seeds in moist sand. Especially noteworthy was the uniform growth of plants and uniformity of time of blossoming in the two chambers. The leaves were unusually large and the internodes elongated, but there was practically no lodging. The plants were all about twenty-three inches high. The treated and control plants were identical in appearance. Also, there were no differences to be observed between the plants growing near the inlet and those near the outlet.

The chambers were alternated between the control and treated sets in the two experiments so that if there were any differences due to conditions in the chambers they would be discovered. There was, as shown, a large increase in dry weight, indicating that the plants grew considerably, and any effect due to the treatment should have been noticeable. As indicated in the last line of table I, the treated plants weighed 1.7 per cent. more in experiment 1, and 2.2 per cent. less in experiment 2 than the controls. There was thus no significant difference apparent from growing in highly ionized air.

#### EXPERIMENTS 3 AND 4

Since the previous experiments show no differences by using bright light, and as HENRICI's results indicated that the effect on assimilation was more pronounced in dim light, it was decided to grow two sets as in experiments

**TABLE I**  
**RESULTS OF EXPOSING BEAN PLANTS TO IONIZED AIR**  
**DATA FOR THIRTY-THREE PLANTS**

	EXPERIMENT 1		EXPERIMENT 2	
	TREATED	CONTROL	TREATED	CONTROL
	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
Fresh weight, tops .....	406	393	375	387
Fresh weight, roots .....	88	95	77	82
Dry weight, tops .....	49.1	47.9	45	46.1
Dry weight, roots .....	6.8	7.1	5.2	5.3
Total dry weight .....	55.9	55.0	50.2	51.4
Weight of 33 seeds .....	10.94			
Weight of 33 seedlings, dry ....	7.3			
Percentage increase during period of treatment .....	766%	753%	688%	700%
Percentage difference of treated	+ 1.7%		- 2.2%	

1 and 2, except that the bright lights (1000 watts) were on for only eight hours and were replaced with 100-watt bulbs for the remaining sixteen hours of each day. Under this changed condition, the plants did not grow as well. They were shorter, the leaves were smaller and seemed less succulent. A large percentage of the blossom buds aborted, the immature blossoms falling off before maturing. There were no apparent differences between the treated plants and controls. The dry weight of thirty-three plants was much less than before (table II). The figures in the table indicate how uniform the conditions of growth must have been.

#### EXPERIMENT 5

Again using ionized air two hours in the morning (9 to 11 A. M.) and two hours in the afternoon (3 to 5 P. M.), and lighting each case with two 1000-watt bulbs, as in experiments 1 and 2, no significant effect was obtained (table II).

**TABLE II**  
**RESULTS USING LIGHT OF LOW INTENSITY AND IONIZATION INTERMITTENTLY**  
**DRY WEIGHTS FOR 33 PLANTS**

EXPERIMENT	TREATED	CONTROL	PERCENTAGE DIFFERENCE
	<i>gm.</i>	<i>gm.</i>	%
3 .....	34.0	33.4	+ 1.8
4 .....	31.0	31.0	0.0
5 .....	47.5	48.7	- 2.5

In the above experiments with ionized air the radon tubes, rather than a spark discharge, were used because the high frequency could not be cut down to a point where the  $O_3$  produced did not kill the plants. Using the blue-white sparks direct from the secondary, with condensers in parallel, it was found that also here, unless the sparking and potential were cut down to a point where relatively very few ions were produced, the ozone was still excessive. Radon produced no detectable  $O_3$ .

#### EXPERIMENT WITH OZONE

In this experiment the procedure was to start with applications of relatively high concentrations of ozone, 1 part in 50,000 to 100,000 of air, in which case results were visible in a few minutes, and then decrease the concentration until either no toxic or no beneficial effects were indicated. Bean plants were used as before.

It was found, first, that concentrations of one part of ozone in 100,000 of air resulted in a wilting of the leaves in 15 to 30 minutes. The lower and older leaves succumbed first, while the very youngest were not affected. The ozonizer was then disconnected and the plants allowed to recover, if still capable of doing so. The wilted leaves did not recover but became bleached, giving a yellow enameled appearance. In those less affected there remained green stripes along the larger veins, while in others the part of the leaf nearest the stalk appeared unaffected.

The topmost younger and not fully expanded leaves were unaffected. The plants were allowed to continue to grow, but, although they bloomed at the same time as the controls, they were stunted and their dry weight, including the bleached leaves, was only three-fifths as much.

With concentrations of one part of ozone in 1,000,000 of air applied only for periods of 30 minutes twice daily, the plants presented a somewhat stunted and dried up appearance, and in eight days brown spots appeared on the older leaves. If this concentration was applied continuously, the plants showed a slightly withered appearance of the leaves and brown spots appeared on the second day.

With a concentration of 1 part in 3,000,000 of air there was no noticeable desiccated appearance of the leaves, and the spotting occurred in four days, always on the older leaves. If the application was continued after this, the spots increased in size and the leaves dried up and fell off, the younger leaves gradually being affected, and then the stem.

With concentrations of 1 part in 6,000,000 of air the spotting did not occur until the twelfth day, the plants appearing normal until then.

With a concentration of 1 part of ozone in 8,000,000 of air, a slight speckling was shown in three weeks.

Another set of plants was grown at a concentration of 1 part of ozone in 10,000,000 of air, applied during two one and one-half-hour periods daily,

and another set at a concentration of one part in 17,000,000 of air continuously for eighteen days. In neither case was there any noticeable change in appearance nor significant difference in the dry weights which were 41.8 and 43.4 gm. for the two treated lots respectively, and 42.9 gm. for the control.

In the above cases of spotting, closer examination showed that the spots originated mostly in the islets between the smaller veins, where there was also a greater density of stomata. The affected cells appeared darkened, desiccated, and collapsed. Similar effects at like concentrations were found for such plants as *Abutilon* and *Coleus*.

Since it was noted that ozone in sufficient concentration had a bleaching action on chlorophyll and it seemed reasonable to assume that such a strong oxidizing agent might accelerate respiratory or aging processes, it was decided to try the effect of different concentrations of ozone on the rate of ripening of green bananas and tomatoes. Results indicated that no change occurred in the rate of ripening of bananas or tomatoes. The conditions were such that the fruit ripened excellently, but unless sufficient concentrations of ozone were used to produce brown spots, no effect was noted. Much higher concentrations of ozone (1 part in 30,000) were required to spot the fruits than was found to so affect the leaves, probably due to the lack of stomata and greater cutinization of the epidermis of the fruits.

The Tesla coil and discharge box being available, it was thought advisable to determine what effect, if any, the Tesla discharge might have on dry and germinated seeds. Consequently dry seeds and germinated seedlings of corn and sunflower were treated for periods of various lengths. The seeds and seedlings were suspended on a heavy sheet of paper between the discharge points of the two plates. Only the fine spray sparks were used and not the cracking ones, also various point distances were used. They were thus subjected to an intense high frequency field and a high concentration of ozone. No effect was noted on the growth of the plants unless the seeds were exposed for as long as 12 minutes at a point distance of 4 cm. and frequency of 4,500,000 cycles per second. This treatment resulted in stunted plants, most of which did not continue to grow after eight to ten days, although none of the seeds were killed outright by the treatment.

### Discussion

The writer thus finds that the results of KOERNICKE, and of LIPPERHEIDE to the effect that ionization of the air promotes growth are not confirmed. The writer used a much higher degree of ionization, both continuously and intermittently, than did LIPPERHEIDE; also, the degree of ionization varied greatly between the entrance and exit, and no effect of ionization gradient was noted. It is not likely that such low degrees of ionization as LIPPER-

HEIDE must have used could have had any effect on growth. His lack of descriptive information makes it impossible to duplicate his conditions. We cannot use increased ionization of the air, therefore, to explain some of the previous results of electroculture but must fall back on criticism of control of conditions.

In regard to ozone, the necrosis of only local areas at first, and these on the older leaves, is difficult to explain. Usually with toxic gases the younger parts are first attacked. Microscopic examination showed that the spotting occurs in areas where the stomata are more numerous. It is suggested that the action of ozone is partly the saturation of lipoid compounds in the cell membranes, resulting in loss of semipermeability, wilting, oxidation of cell contents, and death of the cell.

Since it is difficult to summarize completely the errors in previous work that indicate opposite results to those described in this paper without too much repetition, the reader is referred to the section on results of previous investigators. It may be said here, however, that major mistakes resulted from not isolating the factors, not measuring the conditions, measuring rates of production of ions rather than concentration (actual number per cc.), not controlling other conditions, such as humidity, light, etc., not using a sufficient number of plants, and the use of very weak sources of ionization. The reader is requested to check the methods developed in this paper to assure himself that the technic here used did not admit of any conditions that would invalidate the results.

It may be said that in the experiments herein described plants for the first time have been grown for a period in conditions where the factors of ionization of the air, and ozone, were isolated. The writer contends that examination of growth over a period (from seedling to blossoming) is more likely to show the effects of these factors than measurements of assimilation or respiration over short, intermittent periods.

### Summary

1. Plants grown in highly ionized air (1,100,000,000 ions to 21,000,000 per cc.) showed no significant changes in appearance or dry weight over controls, whether the treatment was applied continuously or intermittently with bright or with dim illumination.
2. Ozone is toxic and produces necrotic spots with final desiccation of the leaf in concentration of 1 part in 1,000,000 to 1 in 8,000,000 of air.
3. Lower concentrations of ozone, which are still easily detectable by odor, produce no stimulation of growth or other effects on the appearance of the plants.
4. Higher concentrations, about 1 part in 100,000, produce prompt wilting and bleaching, the older leaves succumbing long before the younger upper ones.

5. Ripening processes in tomato and banana are not accelerated by  $O_3$ . High concentrations produce spotting. These are much higher than concentrations required for spotting of leaves.

6. A Tesla discharge had no effect on dry or germinated seeds unless exposure was for twelve minutes, when stunted plants that did not develop were produced.

7. A method is developed that would be applicable to a study of the effects of different gases on plants under controlled conditions.

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# INTENSITY OF REMOVAL OF CATIONS FROM COTTON, CORN, AND SOY BEAN TISSUE BY FRACTIONAL ELECTRODIALYSIS<sup>1</sup>

H. P. COOPER, W. R. PADEN, AND R. L. SMITH

(WITH ONE FIGURE)

## Introduction

In a consideration of the characteristics of various plants a number of empirical studies have been made on the soil fertility requirements, shade tolerance, and food value of plants grown under varying environmental conditions (2, 3, 4, 5). There seemed to be some correlation between the relative strength of ions required by plants for optimum growth and the quality of light required. Certain plants which grow successfully in the shade may utilize relatively more of the weak nutrient ions. It might be expected that plants which have the ability to utilize weak nutrient ions and tolerate shade would synthesize organic compounds of relatively low quality energy value. These general observations lead to a consideration of the application of certain of the modern concepts, which have been useful in chemistry and physics, to soil fertility and mineral nutrition studies of plants.

Since every form of energy may be considered as compounded of two factors, one the intensity factor and the other the capacity factor, it is desirable to know the relative importance of these two factors in biological reactions. The data reported are concerned with the intensity factor and only incidentally with the capacity or quantity factor. It is believed that the life processes are more intimately concerned with the intensity factor of energy.

The recent interest in the studies of soil colloids has led to new concepts concerning soil chemistry and the availability of nutrients in the soil. In a recent paper (4) special consideration has been given to the intensity of removal of added cations from soil colloids by electrodialysis. It was found that there is a differential in the intensity of removal of certain cations from soils, and that there is a correlation between the intensity of removal and the strength of ions. The strongest ions were removed most rapidly from the soil. The oxidation-reduction potential values were found to be useful in predicting the intensity of removal of cations from soils by electrodialysis (3, 4, 5). Since there is apparently a correlation between the intensity of removal of some nutrient ions from the soil and the intensity of their absorption by certain plants, one might expect that there would also

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be a differential in the intensity of removal of cations from plant tissue by electrodialysis. In order to secure data on this subject electrodialysis studies were made on the tissue of a number of crop plants.

### Electrodialysis studies of plant tissue

The intensity of absorption of nutrient ions by plants would be expected to be correlated with the intensity of their removal from soil by electrodialysis. It is well known that certain plants differ markedly in the ions which they selectively absorb. As there is such a variety of conditions determining the intensity of absorption of nutrient ions, such as permeability of root membrane, utilization of ions, and formation of insoluble compounds, it is very difficult to determine the order of intensity of absorption of ions by different plants. However, it is believed that fractional electrodialysis studies of plant tissue will serve to establish the general relationship between the relative strength of ions and their intensity of absorption, mobility in tissues, and utilization by certain plants.

In order to determine the intensity of removal of cations, fresh samples of cotton, corn, and soy bean tissue were electrodialyzed in a Löddesöl electrodialysis apparatus (8), and analyses of the diffusate collected at successive intervals for a total of 29 to 32 hours. The usual analytical methods employed in soil studies were used for the determination of cations removed from the plant tissue (12). The uranyl-zinc-acetate method (1) was used in some of the determinations of the sodium content of the diffusate.

The data secured in these studies are shown in table I and in figure 1. The milliequivalents (ml. eq.) removed during each extraction period per 100 gm. of fresh plant material and the percentage of the total milliequivalents for each element are included. The percentage removal of the various metals for each extraction period shows that there is a differential in the intensity of removal of cations from plant tissue.

The percentage of the total amounts of potassium, sodium, calcium, and magnesium extracted from cotton tissue during the first hour period was 91.40, 49.57, 31.10, and 1.60 per cent., respectively. During the 3- to 5-hour extraction period, the above quantitative relations were reversed. The percentage of the total amounts removed was 0.50, 5.18, 11.90, and 60.20 per cent., respectively.

In the studies on corn and soy bean tissue, the first four extraction periods were one-quarter of an hour each. The fifth and sixth periods were one-half an hour each. The percentage of the total amounts of potassium, sodium, calcium, and magnesium extracted from corn tissue during the first quarter-of-an-hour period was 81.55, 22.23, 13.19, and 2.25 per cent., respectively. Likewise the figures for the studies on soy bean tissue were 43.09, 36.47, 1.73, and 1.46 per cent., respectively.

TABLE I

INTENSITY OF EXTRACTION OF CATIONS FROM FRESH COTTON, CORN, AND SOY BEAN TISSUE BY FRACTIONAL ELECTRODIALYSIS IS CORRELATED WITH THE ELECTROMOTIVE SERIES

PERIOD OF EXTRACTION	MILLIEQUIVALENTS PER 100 GM. OF FRESH TISSUE							
	K		Na		Ca		Mg	
hr.	ml. eq.	%	ml. eq.	%	ml. eq.	%	ml. eq.	%
Cotton								
0- 1 .....	7.397	91.40	1.750	49.57	18.360	31.10	0.200	1.60
1- 2 .....	0.459	5.70	0.065	1.84	18.280	31.00	0.280	2.20
2- 3 .....	0.028	0.40	0.116	3.28	8.440	14.40	0.480	3.80
3- 5 .....	0.038	0.50	0.183	5.18	7.000	11.90	7.580	60.20
5- 7 .....	0.002	9.00	0.297	8.41	2.080	3.50	3.180	25.20
7- 9 .....	0.015	0.20	0.189	5.35	1.410	2.30	0.320	2.50
9-11 .....	0.023	0.30	0.207	5.86	1.160	2.00	0.160	1.30
11-13 .....	0.043	0.50	0.146	4.13	0.840	1.40	0.160	1.30
13-21 .....	0.056	0.60	0.342	9.68	0.720	1.20	0.160	1.30
21-29 .....	0.033	0.40	0.235	6.65	0.800	1.20	0.080	6.30
Total extracted .....	8.094	100.00	3.530	100.00	59.090	100.00	12.600	100.00
Corn								
0.00-0.25 .....	2.228	81.55	0.081	22.23	0.592	13.19	0.079	2.25
0.25-0.50 .....	0.451	16.51	0.125	34.72	1.923	42.84	0.039	1.11
0.50-0.75 .....	0.053	1.94	0.053	14.75	1.005	22.39	0.720	20.50
0.75-1.00 .....	.....	.....	0.072	19.98	0.326	7.26	1.174	33.44
1.00-1.50 .....	.....	.....	0.030	8.32	0.209	4.66	0.987	28.10
1.50-2.00 .....	.....	.....	.....	.....	0.107	2.38	0.414	11.79
2- 3 .....	.....	.....	.....	.....	0.128	2.85	0.030	0.85
3- 4 .....	.....	.....	.....	.....	0.122	2.72	0.030	0.85
4- 6 .....	.....	.....	.....	.....	0.056	1.25	0.039	1.11
6- 8 .....	.....	.....	.....	.....	0.021	0.46	.....	.....
8-32 .....	Trace	.....	Trace	.....	Trace	.....	Trace	.....
Total extracted .....	2.732	100.00	0.361	100.00	4.489	100.00	3.512	100.00
Soy bean								
0.00-0.25 .....	2.059	43.09	0.217	36.47	0.173	1.73	0.049	1.46
0.25-0.50 .....	2.236	46.80	0.274	46.05	0.887	8.85	0.020	0.60
0.50-0.75 .....	0.435	9.10	0.074	12.44	2.387	23.81	0.030	0.90
0.75-1.00 .....	0.049	0.01	0.030	5.04	1.387	13.83	0.049	1.46
1.00-1.50 .....	Trace	.....	Trace	.....	1.780	17.75	0.030	0.90
1.50-2.00 .....	.....	.....	.....	.....	1.224	12.21	0.928	27.75
2- 3 .....	.....	.....	.....	.....	0.796	7.94	1.236	36.93
3- 4 .....	.....	.....	.....	.....	0.424	4.22	0.543	16.22
4- 6 .....	.....	.....	.....	.....	0.587	5.85	0.087	2.60
6- 8 .....	.....	.....	.....	.....	0.219	2.18	0.138	4.12
8-10 .....	.....	.....	.....	.....	0.051	0.51	0.069	2.06
10-12 .....	.....	.....	.....	.....	0.036	0.36	0.030	0.90
12-14 .....	.....	.....	.....	.....	0.021	0.21	0.039	1.17
14-16 .....	.....	.....	.....	.....	0.010	0.10	0.030	0.90
16-24 .....	.....	.....	.....	.....	0.030	0.30	0.039	1.13
24-32 .....	.....	.....	.....	.....	0.015	0.15	0.030	0.90
Total extracted .....	4.779	100.00	0.595	100.00	10.027	100.00	3.347	100.00

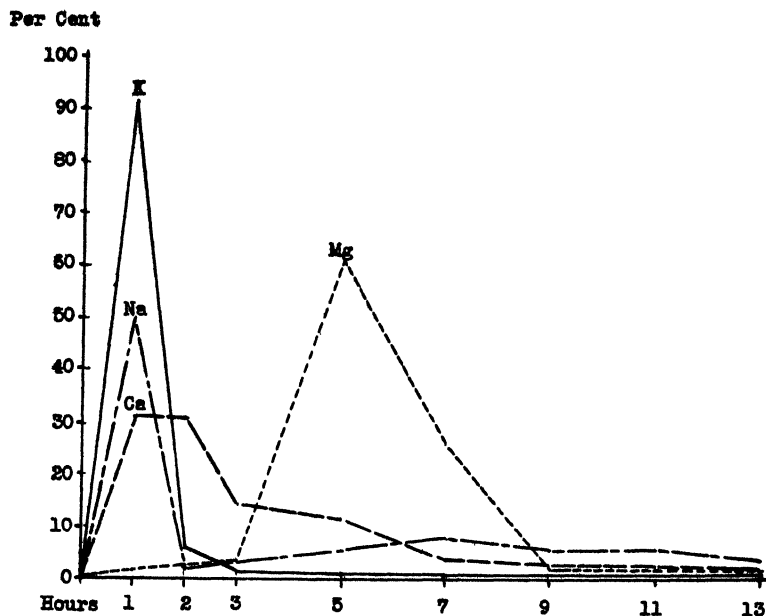


FIG. 1. Percentage of the total milliequivalents of cations removed from 100 gm. of fresh cotton tissue at various intervals by fractional dialysis.

The order of intensity found in these studies is in agreement with the findings of MOORE, REEVES, and HIXON (9), who reported that most of the potassium and sodium was removed from apple tissue during the first hour of electro dialysis. NELLER (10) found that potassium was readily removed from apple tissue.

The data from the fractional electro dialysis studies of plant tissue show the same order of intensity of removal of cations as was found in the soil studies (4). The establishment of the correlation between the oxidation-reduction potential values, the ionization-potential values, and the intensity of removal of cations from soil and plant tissue may be very significant in certain soil and plant nutrition studies. As there is a close relation between the intensity of removal of ions from the soil by electro dialysis and the intensity of their absorption by certain plants, and also the quality of radiant energy required for the assimilation of certain nutrient salts, some oxidation-reduction reactions occurring in growth processes may be of special interest.

#### Some common oxidation-reduction reactions occurring in physiological processes

Since there is apparently a close relation between the intensity of removal of ions from soil colloids and plant tissue by electro dialysis and the relative

strength of ions, it is interesting to consider the significance of these relations in plant nutrition studies. As there is apparently a close correlation between the strength of ions and the intensity of their absorption by plants, it would seem reasonable to expect that there would be a close correlation between the relative strength of ions required by plants for optimum growth, the quality of light required for their assimilation, and the food value or quality of energy supplied by certain organic compounds.

Some of the important energy relationships represented by the light absorption bands of chlorophyll are in close agreement with the free-energy decrease in the formation of certain common nutrient salts, and with the energy required for the reduction of common nutrient anions such as nitrates, borates, carbonates, and phosphates (3).

Most plant life is dependent upon the nutrient salts and the absorption of light energy from the sun. It might be expected that there would be a

TABLE II

SOME COMMON EQUIVALENT OXIDATION-REDUCTION REACTIONS OCCURRING IN CERTAIN PHYSIOLOGICAL PROCESSES WHICH TAKE PLACE ON APPROXIMATELY THE SAME ENERGY LEVEL\*  
APPROXIMATE EQUIVALENT ENERGY IN SOLAR SPECTRUM RANGES FROM 0.41 TO 4.20 VOLT-FARADAYS, WHICH IS EQUIVALENT TO 30,000 TO 2900 ÅNGSTRÖMS

REACTION AND EQUIVALENT ENERGY INVOLVED = VOLT-FARADAYS						EQUIVALENT LIGHT ENERGY		
						ÅNGSTRÖMS	COLOR	
$\text{NO}_3^-$	=	$\text{NO}_2^-$	+	O	+	1.05	11757	Infrared
$\text{H}_2\text{O} + \text{O}$	=	$\text{H}_2\text{O}_2$			+	1.03	11985	Infrared
$\text{CuCl}_2$	=	Cu	+	2Cl	+	1.01	12223	infrared
$\text{Mn}^{++}$	=	$\text{Mn}^{+++}$			+	1.07	11537	Infrared
$\text{BO}_3^{---}$	=	$\text{BO}_2^-$	+	O	+	1.77	6974	Red
$\text{FeCl}_2$	=	Fe	+	2Cl	+	1.78	6935	Red
$\text{H}_2\text{AsO}_4^-$	=	$\text{H}_2\text{AsO}_3^-$	+	O	+	1.98	6235	Orange
$\text{CrCl}_2$	=	Cr	+	2Cl	+	1.90	6497	Orange
$\text{H}_2\text{CO}_3^-$	=	$\text{H}_2\text{CO}_2^-$	+	O	+	2.85	4332	Violet
$\text{MgCl}_2$	=	Mg	+	2Cl	+	2.85	4332	Violet
$\text{H}_2\text{PO}_4^-$	=	$\text{H}_2\text{PO}_3^-$	+	O	+	3.35	3685	Ultraviolet
$\text{CaC}_2\text{O}_4$	=	Ca	+	$\text{C}_2\text{O}_4$	+	3.35	3685	Ultraviolet
$\text{H}_2\text{PO}_3^-$	=	$\text{H}_2\text{PO}_2^-$	+	O	+	3.79	3257	Ultraviolet
$\text{CaCl}_2$	=	Ca	+	2Cl	+	3.85	3206	Ultraviolet
CaHPO <sub>4</sub> decomposition potential .....						4.20	2939	Ultraviolet
Effective rays in preventing rickets .....						4.16	2968	Ultraviolet

\* These values were obtained from International Critical Tables 5: 169-207. 1929.

close relation between the salts used by plants and the quality of radiant energy absorbed by them.

Little attention has been given to the definite correlation between the quality of the free-energy decrease in the formation of nutrient materials and light absorption by plants. Certain data have been compiled to suggest to those interested in photosynthesis studies the possible significance of these relationships in a study of the soil fertility and light requirements of plants and the quality of the energy supplied by certain food materials.

Some of the common oxidation-reduction reactions occurring in nutritional processes are compiled and included in table II. Since the higher plants depend upon energy from the sunlight for growth, it is desirable to consider the energy relationships involved in terms of volt-Faradays, wave length in Ångstroms, and color of light. All of these values can be converted from one to another very easily by the use of Einstein's photochemical equation. This equation and the factors used in conversion of one mode of expression of energy into another were taken from International Critical Tables (7) and RICHTMYER (11), and are as follows:

$$E = Nh\nu = Nh \frac{c}{\lambda}$$

$$\text{Ångstroms} = \frac{12345}{\text{volt-Faradays}}$$

$$\text{Volt-Faradays} = \frac{\text{gram calories}}{23058}$$

$$\text{Gram calories} = \text{volt-Faradays} \times 23058.$$

The conversion of the energy values involved in various reactions into equivalent volt-Faradays, Ångstroms, and color of light shows more clearly the relationship between each of these values.

The reactions which occur on approximately the same energy level, and which may be effective in bringing about certain oxidation-reduction reactions necessary in physiological processes, are arranged in groups. It has been observed that on certain soils relatively high in nitrates there has been a marked response to application of copper, and in some instances to manganese. These responses are very probably related to their effect upon oxidation-reduction reactions. The greatest growth response observed from additions of boron to nutrient media seems to be in complexes where iron may not be readily available. There is also a relation between magnesium chloride and the reduction of the carbonate ion, which is a very important reaction in photosynthesis. Some of the important reactions, such as the reduction of nitrates to nitrites, the formation of hydrogen peroxide, the free energy decrease in the formation of cupric chloride ( $\text{CuCl}_2$ ), and the change

of valence of manganese from  $Mn^{++}$  to  $Mn^{+++}$  are on approximately the same energy level. Likewise there is a close relation between the reduction of the borate ion to the borite ion and the free-energy decrease in the formation of ferrous chloride ( $FeCl_2$ ); between the reduction of the carbonate ion to the carbonite ion and the free-energy decrease in the formation of magnesium chloride ( $MgCl_2$ ); and between the reduction of the phosphate ion to the phosphite ion and the free-energy decrease in the formation of calcium oxalate ( $CaC_2O_4$ ). The decomposition potential of di-calcium hydrogen phosphate ( $CaHPO_4$ ) and the rays effective in the prevention of rickets in animals under certain conditions require approximately the same quality of energy.

It is believed that there is a definite relation between these reactions which take place at approximately the same energy level and that a careful study of these reactions will be of great value in solving some of the complex problems in nutrition. One might consider the close agreement between these values as a simple coincidence and that they need not necessarily bear any significant relationship one to the other. However, the possibility that these reactions may have a definite relationship would seem to merit careful consideration.

As there is a direct correlation between the strength of atomic metallic ions and their removal from soils by electrodialysis as well as their selective absorption by plants, a correlation would be expected between the optimum strength of nutrient ions required by plants, the quality of light required for their normal growth, and their shade tolerance. It is interesting to note that there is a very close correlation between the energy relationships represented by the light absorption bands of chlorophyll, the decomposition or discharge potentials of certain nutrient salts, and the quality of the energy necessary for the reduction of some common nutrient anions such as nitrates, borates, carbonates, and phosphates.

The relationships discussed in this paper are what one might expect from the known physical and chemical properties of the materials considered. A utilization of the modern concept in physics and chemistry to biological problems will make it possible to do more inductive research instead of depending largely upon results secured from empirical methods.

### Summary

1. Samples of plant tissue were electrodialyzed in order to determine the intensity of removal of the various metals. The data presented illustrate very clearly the differential in the intensity of removal of various metals from plant tissue. The strongest ions are removed most readily from the plant tissue. Large proportions of the strong potassium and sodium ions were found in the first fractions of the diffusate. The fractional electrodi-



alysis studies on the intensity of removal of metallic ions from soils and from plant tissue, as would be expected, are in complete agreement.

2. A definite relationship exists between the intensity of removal of nutrients from the soil and the intensity of their absorption by plants. Therefore, it would seem logical to expect a definite relationship between the strength of ions used by plants, the quality of light required for their optimum growth, and the food value or quality of energy supplied by certain organic compounds.

3. Compiled data suggest that there is a relationship between certain common oxidation-reduction reactions and the free-energy decrease in the formation of certain nutrient salts, and also between the energy required for the reduction of certain nutrient anions, such as nitrates, borates, carbonates, and phosphates, and the quality of the light necessary for the optimum assimilation of the various nutrient materials by plants.

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## LEAF TEMPERATURES OF SUGAR CANE

C. F. MORELAND

Since the results in the literature are at considerable variance regarding leaf temperatures with reference to the surrounding air temperature, the writer thought it would be of interest to check some leaf temperatures in a different section of the country from those previously reported. The investigations of CURTIS (2) were performed at Berkeley and Riverside, California, and those of CLUM (1) at Ithaca, New York. The results of MILLER and SAUNDERS (5) were obtained in Kansas, and those of EATON and BELDEN (4) in Arizona.

### Material and methods

The writer's experiments were carried out in the fall of the last year at Baton Rouge, Louisiana. Some of the experiments were performed under field conditions while others were with cut plants in the laboratory.

Sugar cane, variety P.O.J. 213, was used as experimental material. The plants used were in the variety test plots at the Louisiana Experiment Station, Baton Rouge. At the time of the experimentation the plants were 8 to 10 feet high.

Thermocouples made of copper and constantan wires (30 gauge) were used for making the temperature readings. The temperatures were ascertained in two different ways. Under field conditions, the standard junctions of two thermocouples were fastened against the bulb of a thermometer by means of a small rubber band. The thermometer together with the thermocouples was then placed in a thermos bottle filled with water having a temperature about that of the air. The other junction of each thermocouple was placed as follows: No. 1 was passed through the leaf and the wires bent in such a way that the junction was in contact with the lower epidermis of the leaf, or, in some cases, no. 1 was inserted into the mid-vein from the lower side of the leaf. No. 2 was allowed to hang in the shade of the box used to convey the apparatus, or, in some cases, was allowed to hang in the direct sunlight. The two leads from the thermocouples were attached to a multiple switch and thence to a galvanometer (Leeds & Northrup no. 2420 B). With this outfit, the direction and amount of deflection of the galvanometer were tabulated. The temperature of the water in the thermos bottle, being near that of the air, remained quite constant for the duration of each series of readings (about one hour).

After the readings had been obtained (in the field) the galvanometer deflections were then calibrated (in the laboratory) against each thermocouple. The standard junctions were placed in water adjusted to the same

temperature as under the experimental conditions. The other junctions were also fastened to the bulb of a thermometer as the standard junctions. These were placed in a beaker of water, the temperature of which was adjusted at half-degree intervals until the deflections of the galvanometer covered the entire range of the scale. From these deflections a graph was charted for each thermocouple and the deflections under field conditions were translated into temperatures. The accuracy of this method depends largely on the accuracy of the thermometer used, but, as CURTIS (2) has pointed out, the changes in leaf temperatures are so rapid and great that small differences in temperature ( $1/10^{\circ}$  to  $1/20^{\circ}$  C.) are small enough to be of little importance.

With this outfit deviations in temperature of only about  $5^{\circ}$  C. ( $2.5^{\circ}$  above and 2 to  $5^{\circ}$  C. below the standard junction) could be determined. Since differences in temperature were often greater than this, the method was modified and a Leeds & Northrup student type potentiometer was used in conjunction with a standard cell and with the galvanometer described above.

Since this apparatus was not mounted in such a way as to be portable, the top halves of cane plants were cut, placed immediately in water, and then brought to the laboratory. The cut plants were placed directly in front of a large south window which was open from top to bottom. There was no artificial heat being used in the building at the time. Owing to the angle of the sun's rays at the time of the year the experiments were conducted, the plants were well exposed to direct sunlight in the middle of the day.

In using the potentiometer the standard junctions of the thermocouples were placed in a thermos bottle filled with crushed ice and distilled water.

Two sets of temperature readings were obtained under field conditions and three sets under laboratory conditions.

### Experimental results

In the afternoon of October 16, 1936, the weather was clear but very windy. October 23 was again clear but not nearly so windy as the previous week. The results of the temperature readings taken in the field on these two dates are given in tables I and II. It is to be noted that leaf temperatures are more variable under windy conditions than when the air is relatively still; also, that there is a greater difference between the leaf temperature and the surrounding air when the air is still. On October 16, when the wind was blustery, the temperature changes were so rapid that it would have been necessary to take simultaneous readings of leaf and air temperatures in order to make them entirely comparable for a given instance, as the temperature changes were very rapid. The galvanometer deflection would change 10 to 15 mm. ( $1$  to  $1.5^{\circ}$  C.) in one or two seconds.

TABLE I

COMPARISON OF TEMPERATURE OF SUGAR CANE LEAVES AND SURROUNDING AIR UNDER FIELD CONDITIONS ON A CLEAR WINDY (VERY) DAY. RELATIVE HUMIDITY 59 PER CENT.

READINGS 3: 00 TO 4: 00 P.M., OCTOBER 16, 1936. AVERAGE BASED ON 25

READINGS. LEAF TEMPERATURE OBTAINED BY THREADING THERMO-  
COUPLE INTO MIDRIB FROM LOWER SIDE OF LEAF

THERMOCOUPLE NO.	RELATION TO SUNLIGHT		TEMPERATURE		
	DIRECT SUNLIGHT	SHADE	MINIMUM	MAXIMUM	AVERAGE
			°C.	°C.	°C.
1	leaf		31.00	32.50	32.10
2	air		29.50	31.50	31.15
1		leaf	27.20	27.50	27.30
2		air	28.00	28.75	28.30

TABLE II

COMPARISON OF LEAF TEMPERATURES AND SURROUNDING AIR UNDER FIELD CONDITIONS ON A CLEAR AND SLIGHTLY WINDY DAY. RELATIVE HUMIDITY 63 PER CENT. READINGS

3: 00 TO 4: 00 P.M., OCTOBER 23, 1936. AVERAGE BASED ON 10

READINGS. THERMOCOUPLE THREADED THROUGH LEAF  
IN CONTACT WITH LOWER EPIDERMIS

THERMOCOUPLE NO.	RELATION TO SUNLIGHT		TEMPERATURE		
	DIRECT SUNLIGHT	SHADE	MINIMUM	MAXIMUM	AVERAGE
			°C.	°C.	°C.
1	leaf		32.8	33.5	33.3
2	air		27.1	27.5	27.4
2		air	25.1	25.4	25.2

In the laboratory the temperature readings were taken under three sets of conditions. The first set of readings was taken on a clear day between 3:00 and 4:00 P.M. on November 3 (table III). Due to the season of the year and the time of the day, the sun's rays were coming through the open window very obliquely. The next two sets of readings were taken between 11:00 and 12:00 A.M., when the sun was almost directly in front of the window. However, the weather conditions were quite different on these last two days. On November 18, the day was partly cloudy, and sunshine alternated frequently with cloudy conditions owing to small patches of clouds passing in front of the sun. Also at times the clouds were so thin that the sun would partly shine through them (a condition described as hazy in table IV). November 20 was a clear, quiet day. The temperature readings are given in table V.

TABLE III

COMPARISON OF TEMPERATURES OF SUGAR CANE LEAVES AND SURROUNDING AIR IN THE LABORATORY, IN FRONT OF AN OPEN WINDOW, ON A CLEAR DAY. RELATIVE HUMIDITY 86 PER CENT. READINGS 3:00 TO 4:00 P.M., NOVEMBER 3, 1936.  
AVERAGE BASED ON 12 READINGS. THERMOCOUPLE THREADED THROUGH LEAF IN CONTACT WITH LOWER EPIDERMIS

THERMOCOUPLE NO.	RELATION TO SUNLIGHT		TEMPERATURE		
	DIRECT SUNLIGHT	SHADE	MINIMUM	MAXIMUM	AVERAGE
			°C.	°C.	°C.
1 .....	leaf	.. ..	25.15	26.25	25.49
2 .....	air	.. ..	23.75	25.25	24.95
1 .....	.. ..	leaf	23.25	23.50	23.41
2 .....	.. ..	air	24.00	25.00	24.46

TABLE IV

COMPARISON OF TEMPERATURES OF LEAVES OF CUT SUGAR CANE PLANTS AND SURROUNDING AIR IN THE LABORATORY ON A PARTLY CLOUDY DAY. RELATIVE HUMIDITY 63 PER CENT. READINGS 11:00 A.M. TO 12:00 M., NOVEMBER 18, 1936. THERMOCOUPLE THREADED THROUGH LEAF IN CONTACT WITH LOWER EPIDERMIS

THERMOCOUPLE NO.	RELATION TO SUNLIGHT			No. OF READINGS	TEMPERATURE		
	DIRECT SUNLIGHT	SHADED BY CLOUD	HAZY		MINIMUM	MAXIMUM	AVERAGE
					°C.	°C.	°C.
1 .....	leaf	.. ..	.. ..	3	28.25	29.15	28.60
2 .....	air	.. ..	.. ..	3	24.60	26.25	25.70
2 .....	.. ..	air	.. ..	8	22.90	24.90	23.56
1 .....	.. ..	leaf	.. ..	8	22.50	23.50	23.24
1 .....	.. ..	.. ..	leaf	7	23.50	26.90	24.46
2 .....	.. ..	.. ..	air	7	23.40	26.00	24.25

### Discussion

The temperatures found for the sugar cane leaves, as compared with the surrounding air, were more in agreement with the temperature readings obtained by CURTIS and CLUM than with those of MILLER and SAUNDERS and of EATON and BELDEN. The writer did not find leaf temperatures in direct sunlight, however, to be as much in excess of the surrounding air as did CLUM (1) or CURTIS (2), who often found leaf temperatures 10° above the air temperatures. As shown in table V, the greatest average difference observed by the writer was 8.5° C. This difference was even less on a very windy day (4.5° C.). CURTIS (2) has suggested some probable reasons why

TABLE V

COMPARISON OF TEMPERATURES OF SUGAR CANE LEAVES AND SURROUNDING AIR IN THE LABORATORY IN FRONT OF AN OPEN WINDOW ON A CLEAR DAY. RELATIVE HUMIDITY 51

PER CENT. READINGS 11:00 A.M. TO 12:00 M., NOVEMBER 20, 1936.

LEAF TEMPERATURE OBTAINED BY THREADING THERMOCOUPLE

THROUGH LEAF AND IN CONTACT WITH LOWER

EPIDERMIS, OR THREADED INTO MIDRIB

FROM LOWER SIDE OF LEAF

THERMO- COUPLE NO.	RELATION TO SUNLIGHT		NO. OF READ- INGS	TEMPERATURE		
	DIRECT SUNLIGHT	SHADE		MINI- MUM	MAXI- MUM	AVERAGE
				°C.	°C.	°C.
1 .....	leaf (on surface)	.....	15	31.25	38.25	34.91
1 .....	leaf (in midrib)	.....	10	30.00	36.75	33.63
2 .....	air	.....	15	27.75	31.25	29.54
2 .....	.....	air	10	25.75	27.00	26.41
1 .....	.....	leaf (on surface)	10	25.00	26.25	25.61

the data of some workers are at variance with those of others. He points out that the data of different investigators were obtained under different conditions of humidity and that this may influence leaf temperatures through its effect on infrared radiation. He points out also that different methods of obtaining the air temperature were used, and that great changes in the temperature of leaves in direct sunlight may be brought about by changes in rates of air flow. It seems to the writer that these differences in air movements, especially under field conditions, are significant. On October 16, when the wind was brisk, the average difference between the leaf temperature and the air (shade) was about 3° C., with a maximum difference of only 4.5° C. On November 20, when the air was still, the average difference between the leaf temperature and the air (shade) was 8.5° C. or 7.22° C. in the midrib, with a maximum difference of about 12° C. or 11° C. in the midrib. As stated previously, these differences were probably not as great as this for any given instant of time, for with any two contiguous readings of the leaf and air temperatures the greatest difference observed was 6.25° C. There was not a great deal of difference in relative humidity on these two days.

Of course, in comparing these two sets of data it must be borne in mind that the first were obtained under field conditions while the latter were obtained in the laboratory where cut plants were used. While precautions were taken to disturb the transpiration rate of the cut plants as little as possible, there is a possibility that the rate may have been reduced owing to closing of the stomata. The plants showed no signs of wilting for the duration of the experiment and were noted to be in good condition two days after



the experiment. The differences could hardly have been due to difference in relative humidity because the higher temperatures, as compared with the surrounding air, were obtained when the relative humidity was lower. The results obtained under two different conditions in the field on October 16 and 23, also indicate that air movements have more to do with leaf temperature, as compared with the surrounding air, than differences in relative humidity. It is true that the greater differences between leaf and air temperatures were observed with a higher relative humidity; but the difference in the relative humidity of these two dates was only 4 per cent. The average temperature difference between the leaf and air on October 16 (very windy day) was about  $3^{\circ}$  C. while on October 20 (slightly windy) the average difference was  $8.1^{\circ}$  C. As has been pointed out by CURTIS (3), the transpiration rate should have been greater under the condition of the higher humidity because the  $5^{\circ}$ -C. rise within the leaf in excess of that within the leaves under the lower relative humidity should more than offset the 4 per cent. difference in relative humidity. As shown in tables I and II the positions of the thermocouples for obtaining leaf temperature were different. In the first case the thermocouple was inserted into the midrib while in the second case it was in contact with the lower epidermis. This difference in position of the thermocouple may account for about 1 to  $1.5^{\circ}$  C. of the excess of the second readings over the first as compared to the surrounding air. This is shown in table V.

This point is further emphasized by the fact that the difference in temperature between the leaf in the shade and the surrounding air was not very different with the difference in relative humidity.

### Summary

1. Environmental conditions such as air movements seem to alter considerably the sugar cane leaf temperature as compared with the surrounding air. With strong movements of air about the leaf, the difference is slight ( $1^{\circ}$  to  $2^{\circ}$  C.), but in still air the leaf in the sun is several degrees warmer (about  $5^{\circ}$  to  $7^{\circ}$  C.) than the air.

2. Relative humidity may also affect the leaf temperature as compared to the surrounding air but to a lesser extent than the movement of the air about the leaf.

3. The sugar cane leaf in the shade is slightly cooler (about  $1^{\circ}$  C.) than the surrounding air.

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## BRIEF PAPERS

### SEASONAL VARIATION IN ELONGATION OF YOUNG SEEDLING RADICLES

In October, 1935, we made determinations of the elongation during a twenty-four hour period, at optimum temperature, of the young seedling radicles of lentil, cress, and sunflower. When we repeated the tests in January 1936, we found the elongation in each instance to be decidedly less. But another series of tests made in May 1936—using, of course, seed from the same lots—gave an elongation equal to or greater than that observed the previous October.

#### Methods and results

Seeds of lentil (*Lens esculentum* L.), of cress (*Lepidium sativum* L.) and of sunflower (*Helianthus annuus* L.) were germinated on strips of blotting paper disposed vertically along the inside wall of a metal moist-chamber and kept wet by capillarity downwards, according to the method previously described by one of us (1). When the young radicles were about 5 cm. long, the seedlings, still attached to the strips of blotting paper, were placed with the radicles suspended vertically along the inside wall of a moist chamber consisting of a 500-cc. pyrex beaker (tall form) covered with a petri dish, and this again placed in a 6 x 9-inch battery jar covered with a glass dish. Water on the bottom of beaker and battery jar, and wet blotting paper along the inside wall of each, insured the maintenance of atmospheric humidity close to saturation. Strips of paper over the upper edge of beaker and of battery jar kept the glass cover in each case slightly raised, and permitted circulation of air.

The moist chambers containing the seedlings were placed in a metal-lined incubator with adjustable temperature constant to  $\pm \frac{1}{2}^{\circ}$  C. A fan within the incubator chamber promoted circulation of air and rapid adjustment of temperature. The test period was twenty-four hours. At the start of the

TABLE I  
ELONGATION OF YOUNG SEEDLING RADICLES IN MOIST CHAMBER

PLANT	TEMP.	AVERAGE ELONGATION IN 24 HOURS		
		OCT. 1935	JAN. 1936	MAY 1936
	$^{\circ}\text{C.}$	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>
Lentil	28	27	19	34
Cress	28	33	20	40
Sunflower	32	66	35	60

test the paper strip bearing the seedling was in each instance cut at the length of the radicle; the extension of the radicle beyond the cut end of the strip at the end of the test period was, therefore, a measure of its elongation during that period. The figures in the following table represent, in each case, the average of not less than 30 measurements.

### Discussion

HOAGLAND (2) has noted a marked seasonal variation in root growth of barley plants in solution culture, which he is inclined to ascribe to seasonal differences in quality or intensity of illumination. Likewise, WHITE (3) has observed a seasonal fluctuation in the growth rates of excised tomato root tips, which he considers explainable by variations in laboratory temperature. But our determinations of amount of elongation of radicles in a twenty-four hour period were made in a fairly light-tight incubator in which the set temperature was maintained to  $\pm \frac{1}{2}^{\circ}$  C., so that the seasonal variations in amount of elongation noted by us cannot well be attributed to differences of illumination or of temperature during the test period.

Instead, we are inclined to associate them with the seasonal differences in ventilation conditions in the laboratory. Doors and windows are kept closed more during the winter months; and consequently, it is to be expected that during the cold weather season the air in the laboratory tends to contain higher concentrations of the vapor of compounds contained in, or associated with the use of illuminating gas, paints and oils, varnishes, lacquers, and the like. We have found a number of such substances, even when in extremely low concentration in the air, to have marked influence on the elongation of young seedling radicles.—M. A. RAINES and C. W. TRAVIS, *Howard University, Washington, D. C.*

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APPLICATION OF THE CHLORATE METHOD FOR DETERMINING NITROGEN TO LIGHT, FLUFFY MATERIALS<sup>1</sup>

In using the chlorate method<sup>2</sup> for determining total nitrogen in plant tissues, it was found that certain coarse, fluffy materials tend to float on the acid and to be carbonized, out of contact with the oxidizing reagent. This causes loss of nitrogen. The method, as previously described gives satisfactory results on green vegetative and woody tissue, since they are heavy and stay in the oxidizing solution. Further work on the method shows that fluffy materials, such as dry grass, must at all times be in contact with excess of oxidizing reagent during the heating period, or oxides of nitrogen are likely to be lost.

To make the method reliable for a wider range of materials and to make it unnecessary to grind very fine, the following modified procedure is recommended:

Put 100 to 300 mg. of dry tissue, or 500–1500 mg. of green tissue, into a 200-cc. Erlenmeyer flask. There should be at least 0.5 mg. of nitrogen in the sample used. Add 2 cc. of 50 per cent. sodium chlorate solution for each 100 mg. of dry sample, or each 500 mg. of green sample. Mix well with the sample and allow to soak a few seconds. Add 25 cc. of 52 per cent. by volume sulphuric acid and attach the flask to a water-cooled reflux condenser. Arrange the connections to the condenser in such a manner that the flask can be shaken readily. Heat rather slowly at first. When the reaction begins to become active, shake to wash down particles from the sides. Do not allow blackening of particles on the sides of the flask if it can be helped. However, if the particles of samples have been soaked with a 50 per cent. chlorate solution some blackening does no harm. Heat until the sample is completely gone (2–3 minutes). Stop the heating while there is still a very faint yellow chlorine color. Flush out the condenser with two successive 5-cc. portions of 52 per cent.  $H_2SO_4$ , and cool the flask in a cold water bath. After detaching, cool to room temperature.

Make the cooled solution up to a convenient volume. If the quantity of nitrogen present is small, the volume should be as small as possible. Immediately put exactly 1 or 2 cc. (use an accurately calibrated pipette) of the solution into a 25-cc. test tube and add 3 or 6 cc. of phenoldisulphonic acid, mix well, and allow to stand about  $\frac{1}{2}$  minute. (If a very reddish solution forms here, the heating was not continued long enough to decompose excess chloric acid. The solution should remain almost colorless.) Wash into a

<sup>1</sup> The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

<sup>2</sup> EMMERT, E. M. Method for quickly determining nitrogen in plants, and soluble nitrogen as a measure of the nitrogen available for anabolic processes. *Plant Physiol.* 10: 355–364. 1935.

200-cc. Erlenmeyer with about 10 or 20 cc. of water and make alkaline with 40 per cent. NaOH solution, adding it until the maximum yellow color is produced and a small excess of alkali is present. Make to a volume which brings the yellow color near to the standard. After thorough mixing, the clear solution is compared with a standard (1 cc. = 0.0025 mg. N) in a colorimeter.

### Discussion

Soaking with chlorate solution causes all particles to be completely oxidized without loss of oxides of nitrogen. Close checks with the Kjeldahl method were obtained with all types of samples tried so far, except pyridine and quinoline; but even the Kjeldahl method does not give accurate results with these compounds. Quite coarse samples are digested rapidly. A very important point is to cool the solution to room temperature after digestion and before adding the phenoldisulphonic acid. In hot solutions there is danger of loss of nitrosyl chloride, and even oxides of nitrogen, but there is no loss if the reaction between phenoldisulphonic and nitric acids takes place at room temperature. This point was not emphasized in the previous description of the method, and loss of nitrogen oxides may cause low results if precautions to cool to room temperature are not taken.

The following table presents results obtained on fluffy, coarse samples:

	Chlorate method	Kjeldahl method
1. Bluegrass .....	2.22	2.10
2. " .....	2.80	2.91
3. " .....	2.59	2.64
4. " .....	2.20	2.15
5. " .....	2.15	2.16
6. Oats .....	3.70	3.68
7. Alfalfa .....	3.81	3.74
8. Tobacco .....	5.03	5.05

Each of the determinations reported in the table was completed in less than ten minutes by the chlorate method, while the Kjeldahl method required 6 to 8 hours. Several Erlenmeyers, a reflux condenser, a pipette, test tubes, and a colorimeter are all the apparatus needed for the chlorate method; whereas the usual digestion shelves, distillation equipment, and burettes are needed for the Kjeldahl method. The reagents and standards needed for the chlorate method also are simpler to prepare.

If samples of plant extracts larger than 0.5 cc. are run for soluble nitrogen as previously described the precaution of cooling the solution before adding phenoldisulphonic acid must be exercised. On samples of 0.5 cc. or less, the shaking and blowing out of the chlorine usually cools the solu-

tion sufficiently; but it is best to allow the solution to cool even in smaller samples.—E. M. EMMERT, *Kentucky Agricultural Experiment Station, Lexington, Kentucky.*

### CHLOROPHYLL IN SULTANINA GRAPES AND RAISINS

During ripening, the Sultanina or Thompson seedless grape changes from a green to an amber color. After treatment with sulphur dioxide and drying, the resulting raisin varies in color from amber with a greenish tint, to amber, and dark brown. In this paper is presented evidence identifying the green coloring matter with chlorophyll. The concentration of chlorophyll is so low, (see table I) that the figures given, particularly for the raisins, cannot represent much greater accuracy than that of the correct order of magnitude. The extracts of the sulphured raisins, furthermore, were markedly less stable than those of the fresh grape, and were decolorized in the course of a few hours of exposure to light.

The persistence of unaltered chlorophyll in the raisin may be explained on the assumption that it is inaccessible to the organic acids and to sulphur dioxide, which is known to be largely bound in organic complexes, presumably sulphonic acids. The pigment *in situ* might therefore be expected to survive intact. Extraction, whereby pigment and sulphur dioxide may be brought into the same phase, would lead to the ultimate destruction of the chlorophyll, particularly when exposed to light.

In the destruction of chlorophyll by light it is uncertain whether the process in its early stages is hydrolytic or oxidative or a combination of both, and the absence of well defined colored degradation products *in situ* (with one exception, phaeophytin) has rendered well nigh impossible studies of the reactions involved. Nor can much be said as to the effect of sulphur dioxide on chlorophyll. It is known that the primary effect of weak acids, including sulphur dioxide, is the loss of magnesium, with the formation of phaeophytin (1, 3). Subsequent loss of pigment may involve reactions having nothing to do with the sulphur dioxide.

In the grape and raisin extracts examined, no absorption band with a maximum at 5350 Å was observed, indicating the absence of phaeophytin. Experiments to be reported later indicate a definite reaction rate for the conversion of chlorophyll to phaeophytin, which the writer is measuring, in collaboration with M. A. JOSLYN.

The transmissions of the various chlorophyll solutions from grapes and raisins were measured spectrophotometrically at the position of maximum absorption for the characteristic red band. When compared with standard chlorophyll solutions, and with leaf extracts, it was found that the maximum was apparently shifted 60 Å toward the red. The solutions were then compared with a chlorophyll solution of such dilution that at their apparent



maxima, 60 Å apart, they had identical transmission values. When these solutions were inserted into the two light paths of the spectrophotometer, the displacement of the whole band was plainly visible, for both grape and raisin extracts, with respect to that of the standard. Less subjective methods indicate the shift of band is real, that of the maximum illusory, owing to material change in the skewness of absorption.

The most obvious explanation is that the ratio of the components, chlorophyll *a* to chlorophyll *b*, is markedly greater in these extracts than that found by WILLSTÄTTER and STOLL (4) for normal green leaves, where the ratio is somewhat less than 3:1. An examination of the individual absorption curves for the two components as given by WINTERSTEIN and STEIN, (5) and by ZSCHEILE, (6) shows clearly that the effect of increasing the ratio must be to translate the band further toward the red for a given photometer setting, and to increase the skewness. Additional references on the values of this ratio will be found in the work of WILLSTÄTTER and STOLL (*loc. cit.*), and of INMAN, (2).

### Experimentation

Observations were made on two lots of grapes, those with a definitely greenish tint and those amber in color. The grapes, in lots of 1.2 kg., each were crushed, dehydrated with acetone, and extracted with the same solvent by steeping overnight. The acetone extracts were filtered on a Buchner funnel, the pulp being leached with fresh solvent. The acetone extracts (2 liters) were transferred by successive additions to a smaller quantity (200 cc.) of re-distilled hexane (b. p. 66–68° C.) each addition being followed by removal of the acetone with water. In this way, concentration of the solution was effected without recourse to heating. The solutions were dried over anhydrous sodium sulphate, decanted, and with rinsings made to volume (250 cc.).

Raisins were sorted into three groups: (a) greenish tint, (b) amber, (c) dark brown. The raisins, (0.7 kg.), were passed through a food chopper, and thoroughly mixed with acetone, as in the previous case. After extraction, steeping with fresh solvent yielded no further coloring matter. The acetone extract from the dark brown raisins yielded no pigment, and, therefore, was discarded.

The hexane solutions were in all cases yellow to greenish yellow. Absorption maxima, determined visually, were apparently at 6710 Å, and for chlorophyll (5 X, American Chlorophyll Company), and grass extract at 6650 Å  $\pm$  15 Å.

It was assumed that the absorption coefficients for the two would be equal at their apparent visually determined maxima. This introduces an error since the coefficient for pure *a* is greater than that for an approxi-

mately 3:1 mixture of *a*:*b*. In view of the low concentrations present, more precise measurements were not justified. Spectrophotometric comparisons were then made, with all instrumental factors kept constant. The chlorophyll concentrations were thus calculated, in mg./kg. actual and dry weight, assuming average values of 75 per cent. moisture for the grapes, 15 per cent. for the raisins. The results are given in table I.

TABLE I  
CHLOROPHYLL IN SULTANINA GRAPES AND RAISINS IN MG./KG.

MATERIAL	ACTUAL WEIGHT	DRY WEIGHT
<i>Grapes</i>		
Greenish	1.2	5.0
Amber	0.5	2.0
<i>Raisins</i>		
Greenish	0.5	0.6
Amber	0.35	0.4

If we take as an average, for green leaves, a chlorophyll content of 8 gm. per kg. of dried leaves, it is seen that chlorophyll in mature grapes is less than  $10^{-3}$  times that occurring in leaves.

Small quantities of other acetone extracts were transferred to ether and the following tests were made. The Molisch phase test was unsatisfactory for raisin extracts, presumably owing to the presence of sulphites, though a brownish phase appeared. The yellowish green color of the original solution made this of doubtful value in all cases.

No coloring matter was extracted from ethereal solutions of any of the samples with 22 per cent hydrochloric acid.

N/100 alkali extracted the pigment from ether only in the case of sulphured extracts not freshly prepared, indicating the formation of free carboxyl groups.

### Summary

Extremely low concentrations of chlorophyll have been found in Sultanina grapes and raisins. The evidence indicates that the ratio of the two components, *a* and *b*, is greater than that of normal green leaves, and that the chlorophyll is substantially unaltered *in situ*, but decomposes rather rapidly, on extraction, if the original has been treated with sulphur dioxide.—G. MACKINNEY, *University of California*.

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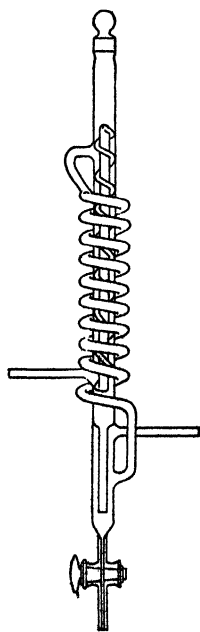
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## THE QUANTITY OF ETHYLENE PRESENT IN APPLES<sup>1</sup>

(WITH ONE FIGURE)

A rapid chemical method for the determination of ethylene in plant tissues has been devised. Its main details follow: 250 gm. of tissue are placed



in a 500-ml. flask together with 100 ml. of water. A reflux condenser, to the upper end of which is attached an evacuated gas sampling tube of 330-ml. capacity, is connected to the flask by means of a ground glass joint. Connection is established between the tube and the flask, and the water and tissue in the flask are boiled until the tissue has broken down, and all the gases present in the flask and tissue have been driven into the gas sampling tube. The tube is then detached, and the gases contained therein are passed over sodamide to remove interfering substances such as alcohol, acetaldehyde, and esters, and finally through 5 ml. of 0.01 N potassium permanganate solution in an absorption unit of special design (fig. 1) which permits 5 minutes contact with the absorbent when the gas is passed through at the rate of 5 cc. per minute. Ethylene is estimated by pipetting an aliquot of the absorbent into excess ferrous sulphate, and titrating the unoxidized ferrous ion with 0.002 N permanganate. Under the conditions of the procedure, the reduction of permanganate is 8 per cent. over the value calculated for the

oxidation of ethylene to ethylene glycol.

<sup>1</sup> Published with the permission of the Director of the Minnesota Agricultural Experiment Station as Scientific Journal Series Paper no. 1436.

Using this procedure, determinations of the ethylene content of Mac-Intosh apples after nine months of storage have been made. These showed an ethylene content of the order of 0.12 mg. per kilogram of fresh tissue.

Calculations from the data of DENNY and MILLER<sup>2</sup> show that they estimated, by the study of epinastic responses induced by apple tissue, an ethylene content of about 0.2 mg. per kilogram of apple tissue, which is in fair agreement with the present results.

It is interesting to note that, expressed on a volume basis, this figure represents an ethylene content of about 1:10,000 in the tissue, which is equal to the concentration calculated to be present in fruits ripened artificially in a chamber in which the ethylene in air concentration has been adjusted to 1:1000, the concentration commonly used in this practice.

Further research on the relation between ethylene content and keeping quality of apples is being conducted at this laboratory.—R. C. NELSON, *Minnesota Agricultural Experiment Station*.

### A TEACHERS' FORUM

At the Kingston meeting of the New England section, the closing session was devoted to a seminar on the teaching of plant physiology. About 40 teachers followed closely a lively discussion of the subject: "What are the logical objectives and methods of approach in teaching plant physiology?". The group found the teachers' problems so important and complex as to warrant further consideration of them at future meetings of the section.

This seminar on teaching methods was an innovation, designed to balance the program of an annual meeting with the interests of the society membership. At the request of the local committee for the Rhode Island meeting, it was organized and conducted by a committee of teachers with Dr. C. J. LYON of Dartmouth as chairman. The basic thought was to make the instruction in elementary physiology more effective and of more real value to college students.

The forum was opened with statements of views prepared by teachers in the two types of institutions, liberal arts colleges, and state universities. Dr. GEORGE P. STEINBAUER of Maine showed how students in his courses came prepared in botany and chemistry and in search of detailed information bearing on their future work in such fields as forestry and horticulture. The written statement of Dr. H. E. PULLING of Wellesley, read by Dr. W. REI ROBBINS of New Jersey, emphasized the necessity in liberal arts colleges of teaching plant physiology as a necessary, important part of biology, and as a basis for understanding all life in relation to its environment.

<sup>2</sup> DENNY, F. E., and MILLER, LAWRENCE P. Production of ethylene by plant tissue as indicated by the epinastic response of leaves. *Contrib. Boyce Thompson Inst.* 7: 97-102. 1935.

The program continued with a demonstration of teaching a topic, by two different methods—the plain lecture full of textbook facts, *versus* demonstration interspersed with questions and references to the personal experiences of the class members. Dr. LINUS H. JONES of Massachusetts State College and Dr. DOROTHY DAY of Smith cooperated in this difficult assignment and with great success.

The open discussion which followed centered on these contrasting classroom methods and their relation to laboratory instruction, prerequisites to physiology courses, teacher personality, and the general objectives of courses. There was a general agreement on the principle of using the lecture-demonstration method for qualitative work, combined, if possible, with quantitative studies in the laboratory. The relative unimportance of specific method, in contrast with the ability and personal qualities of the teacher, was left as an unchallenged observation. The question of requiring training in organic or physical chemistry previous to the course in plant physiology brought out the great differences in the status of physiology courses in universities and liberal arts colleges. Though admittedly desirable for teaching the basic facts of the science, a chemistry requirement is probably impossible in institutions which train primarily for society, or for non-scientific professions. The other questions and answers emphasized the need for each teacher to work out the method and procedure best suited to the individual school and instructor.

Within the time available for a single session, the group could not consider the details of teaching even a single phase of physiology along with the general features. In the words of one teacher present: "It was generally believed that this type of discussion should pave the way for consideration of specific material in the general course in plant physiology."

The exact subject to be taken up next year will be determined by the needs of the members but it is probable that instruction in such new or difficult topics as auxins, translocation, or osmotic relations will be chosen. The undersigned has been requested to organize the teachers' interests within the New England area and will welcome suggestion and other aid in carrying out the assignment.—CHARLES J. LYON, *Dartmouth College, Hanover, N. H.*

## NOTES

**Indianapolis Meeting.**—The fourteenth annual meeting of the American Society of Plant Physiologists will be held at Indianapolis on December 28–30, 1937. The Hotel Claypool has been designated as headquarters of the society, as well as its meeting place, a most convenient arrangement.

As usual, the annual dinner will be held on the evening of the first day of the meeting, December 28. For many years the annual dinner has served as the time and place of announcements of awards of honors, election of corresponding members, the HALES addresses, memorials, etc. It has become the most important and interesting phase of our social contacts. Members and friends are reminded to obtain tickets promptly on arrival, at the time of registration with A.A.A.S. The dinner has always been open to all who cared to attend and enjoy the festivities.

Symposia and joint meetings have been arranged, and there will be the customary sessions for reading brief papers. On Thursday evening, there may be a session devoted to the problems of successful teaching of plant physiology. The success of the New England Section with such a meeting encourages the hope that there may be equal interest in a discussion of methods of presenting our material to students, and that a round-table conference following the discussion of the problems may aid in the improvement of our methods. This session for teaching problems will close the fourteenth annual meeting.

Every member in the middle west will be expected at the meetings, and as many as can come from more distant points. Especially it is hoped that all members of the executive committee may be present to consider important items of business and policy.

**Early Payment of Dues.**—The importance of paying dues early may not be apparent to all of our members. It has been customary to discontinue all memberships at the close of the calendar year, until they are renewed by payment of dues, to reduce losses from sending out copies of *PLANT PHYSIOLOGY* to those who do not intend to renew their memberships. The loss of copies of even one number of the official journal breaks volumes that are held in reserve for future members and subscribers. They are equivalent to cash assets. Moreover, when dues are paid between October 1 and December 31, the secretary-treasurer can retain the addressograph labels without danger of errors, and he can avoid the costs of replacement of labels which follow the discarding of them owing to late payments. Early payment also helps the editors to gauge their use of manuscripts, for they can then know early about what the probable income of the society will be for the ensuing journal

year. Cooperation of members and subscribers in this effort to avoid losses, errors, and additional costs will be greatly appreciated.

**Charles Reid Barnes Life Membership Committee.**—The committee appointed by the president of the society to select the recipient of the thirteenth award of the CHARLES REID BARNES life membership, in the American Society of Plant Physiologists is as follows: Dr. H. R. KRAYBILL, Purdue University, chairman; Dr. ELBERT T. BARTHOLOMEW, University of California; Dr. BERNARD S. MEYER, Ohio State University; Dr. FRANK B. WANN, Utah Agricultural Experiment Station; and Dr. FELIX G. GUSTAFSON, University of Michigan.

**Program Committee.**—The program committee always bears a heavy responsibility in connection with the meetings of the society. For the Indianapolis meeting, the committee is composed of the following members: Prof. R. B. WITHROW, chairman, Dr. J. D. SAYRE, and Dr. W. REI ROBBINS. Everything that can be done for the comfort, convenience, and entertainment of those who attend the annual meeting is being done with efficiency and dispatch. This loyal and faithful service is greatly appreciated by all concerned.

**Executive Committee.**—The membership of the society is entitled to know who constitute the personnel of the executive committee. There are twelve members, five *ex officio*, three elected, and four representatives of the organized sections. The full membership is as follows: Dr. OTIS F. CURTIS, chairman; Dr. R. B. HARVEY, Dr. WALTER F. LOEHWING, Dr. F. P. CULLINAN, Dr. C. A. SHULL, Dr. D. R. HOAGLAND, Dr. H. R. KRAYBILL, Dr. B. E. LIVINGSTON, Dr. B. E. GILBERT, Dr. D. M. DOTY, Dr. H. F. CLEMENTS, and Dr. R. W. LORENZ.

**Chemical Methods.**—A number of workers have found the first supplementary report of the Chemical Methods committee a satisfactory substitute for the original recommendations. As the original report is no longer available, the supplementary report is the only one that can be supplied. Many of our members have come into the society since these reports were published. The first supplementary report, with cover, costs only 15 cents, postpaid, or may be purchased in lots of eight for \$1.00, if as many as that can be used. Those who may care to purchase a copy of the recommendations are advised to write to Dr. W. E. TOTTINGHAM, chairman of the committee, Agricultural Chemistry Building, University of Wisconsin, Madison.

**Errata.**—The customary list of errata found in the first three issues of volume 12 of PLANT PHYSIOLOGY is presented at the close of the table of con-

tents. If these errors are recorded in your copies of the journal you will be protected against the use of erroneous information at some future time. Assistance of all authors is requested to make these lists as complete as possible.

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**Vladimir Nikolaevitch Lubimenko.**—The joy anticipated in presenting the portrait of the great Russian plant physiologist, LUBIMENKO, as a memento at or near his sixty-fifth birthday has been turned to sorrow by his death from a heart attack on September 14, 1937. He had proof-read and returned the short paper which opens this number of *PLANT PHYSIOLOGY*, but could not live to see it in published form. Dr. LUBIMENKO was a corresponding member of the American Society of Plant Physiologists, having been honored by election at the Pittsburgh meeting in 1934.

A few of the significant facts concerning his life and activities are presented here. Born on January 18, 1873, in the government of Voronej, on the frontier of Great Russia and the Ukraine, he grew up and was given his first education among his home surroundings. Formal education began as a teen-age boy at the School of Agriculture at Kharkov, which he attended from 1886 to 1892. At the age of 21 he entered the High School of Forestry at St. Petersburg, where he received a gold medal for his first work on plant anatomy. The interest aroused in these early years led him to specialize in botany, and he was assistant to the professor of botany, Dr. I. BORODIN, while yet a student in the High School of Forestry. He took up additional work at the University of St. Petersburg, where he obtained his diploma in 1902.

Following this work he enjoyed work in foreign lands. In 1903 his institution (High School of Forestry) sent him to Bonn, where he worked on cytology under STRASBURGER, and early the following year he went to the Sorbonne at Paris, for some work with BONNIER. In 1905 he obtained a commission from the Department of Forestry to study the influence of light on trees; he continued on at Paris, engaged in this work for three years.

Returning to Russia in 1908, he entered the service of the Botanical Gardens of Nikita in the Crimea, where he held the position of botanist for five years. He was awarded a master's degree in 1910 for his studies of photosynthesis, and in 1917 his doctor's degree for a major contribution on plastid pigments. In 1913 he again left his native land for foreign study and travel. He undertook a scientific journey to Australia and the Malay Islands, working a part of the time at the Buitenzorg Laboratory in Java. After this journey he began his long service for the Botanical Gardens at St. Petersburg, where he spent the remainder of his life. Invited there as botanist of the Museum, he was soon named director of the laboratory of plant physiology, which was created by him. When, after the revolution, a department of experimental botany was founded, LUBIMENKO became its director.



He served as special lecturer in various institutions, in St. Petersburg at the High School of Lesheft, 1916–1928; at the High School of Geography, later transformed into a Geographical Institute, 1916–1925; at the Chemical-Pharmaceutical Institute, 1922–1930; and at the Military Academy, 1924–1930.

Many honors came to him in recognition of his distinguished services. In 1913 his studies on chlorophyll in collaboration with MONTVERDE were recognized by the award of a prize by the Academy of Science, and again in 1918 the Academy of Science awarded him a prize for his studies on the transformation of the pigments of plastids in the living tissues of plants. It was at this juncture that he was called to the Scientific Institute of Leshaft where he continued his work to the end. In 1922 the Academy of Sciences U.S.S.R. elected him a corresponding member. He was a delegate of the U.S.S.R. at the International Congress of Protection of Nature Monuments at Paris in 1923, and during the summer of that year gave a series of lectures on plant physiology at the University of Paris. In 1925, having been sent again to France, he studied the physiology of Mediterranean algae at the Biological Station of Banijuls sur Mer. He was a delegate from the Academy of St. Petersburg to the fourth International Botanical Congress at Ithaca in 1926, and took an active part in the work of the Congress at the request of the Committee of Organization. He contributed a valuable paper on chlorophyll and the genesis of the photosynthetic apparatus of plants. In 1927 he became a member of the American Society of Plant Physiologists, in 1928 a member of the American Genetic Society, in 1935 a member of the oldest botanical society in France, La Société Linnéenne de Lyon. The Academy of Science of Ukraine elected him as a member in 1929, and since that time he had served as director of its Department of Chemical Plant Physiology. In Russia he had been for many years a member of the Society of Naturalists and the Botanical Society.

His career as a scientific contributor began in 1900, and he was the author, either alone or jointly, of scores of papers which have enriched our knowledge of the physiology of plants. The end of his rich and fruitful life came swiftly, and apparently without warning, on September 14; for up to an hour before his death, he was writing at his writing-table.

In her bereavement we extend to his widow, INNA, our deepest sympathy. The high regard and esteem which her husband had attained personally and professionally in all lands, will be a precious memory to her.

**George Perkins Clinton.**—In the death of Dr. GEORGE PERKINS CLINTON, botanist of the Connecticut Agricultural Experiment Station, on August 13, 1937, the American Society of Plant Physiologists has lost one of its distinguished members. Although primarily a mycologist and pathologist, Dr. CLINTON was keenly interested in related botanical fields, and in botany as

a whole. He was born at Polo, Illinois, May 7, 1867, and had recently celebrated his seventieth birthday anniversary. His interest in agricultural pursuits and the pleasures of botanical collecting may have been stimulated by his early environment. His father was a rural journalist who had won honors from the College of Agriculture at the University of Illinois for his service to rural interests. As a boy GEORGE grew up in the atmosphere of a rural newspaper plant, and in a home where love of rural life was predominant. It was during this period that he began collecting herbarium specimens, a hobby that continued as a serious but most pleasant part of his vocation, throughout life.

His early collegiate training was received at the University of Illinois, at which institution he was awarded his B.S. degree in 1890, and M.S. degree in 1894. Later he entered Harvard, from which he received the M.S. degree in 1901, and Sc.D. in 1902. During this twelve-year period of training, he was assistant botanist at Illinois, and in the Illinois Agricultural Experiment Station. In 1902 he was called as botanist to the Connecticut Agricultural Experiment Station. Here he spent 35 years in mycological and pathological research, with considerable attention to the Ustilagineae, and accomplished much in the warfare against harmful fungal parasites of crop plants and forest trees. He also served as lecturer in forest pathology at Yale University from 1915 to 1926, and as research associate in botany at Yale from 1926 to 1929.

Among his important services may be mentioned his work in Porto Rico, where he was sent in 1904 to study coffee rust; his work at Harvard in 1908 on brown tail moth control; his expedition to Japan in 1909 to obtain parasites for control of the gypsy moth; his work on tobacco wild fire; and his travels for collection of fungi in Canada, Europe, Hawaii, Japan, Porto Rico, and Panama. His valuable collections of specimens, and his library of reprints were presented to the Experiment Station. The departmental library, and these fine collections of *exsiccati* constitute a monument to his foresight and wisdom in the management of his department.

He was a member of the New England Botanical Club, the Botanical Society of America, and many other scientific organizations. He had been honored as vice-president of Section G, A.A.A.S., in 1915; as president of the Phytopathological Society in 1912; as a fellow of the American Academy of Arts and Sciences; and as a member of the National Academy of Sciences.

He is survived by his wife, who lives in New Haven. Their only child, a son, was killed in France. All members of the American Society of Plant Physiologists join in extending heartfelt sympathy to Mrs. CLINTON in her loss.

**Biochemistry of Cellulose, the Polyuronides, Lignin, etc.**—A monograph of much more than usual interest and value is this volume by A. G.

NORMAN, biochemist at the Rothamsted Experimental Station. The development in recent years of new methods of studying the fundamental units of cellulose and other polysaccharides has brought about a much better understanding of the wall materials of plant cells than was possible even a few years ago.

There are eight sections to the volume, dealing with the following subjects: cellulose; polyuronide hemicelluloses; pentosans, hexosans, and hexopentosans; pectin; gums, mucilages, and gel-forming substances; lignin; metabolism of plant cell-wall constituents; and microbial polysaccharides. An appendix is devoted to the discussion of uronic acids and pentoses.

The value of this work is the insight which it permits regarding the biochemical processes involved in the formation of these materials during metabolism. In this sense it is a valuable contribution to true biochemistry, and not a mere discussion of the properties of biochemically produced plant products. The book is highly commended to all students of chemical plant physiology. Dr. NORMAN has presented his material with great skill.

The book contains 232 pages, 12 illustrations, author and subject indexes. It is published by the Oxford University Press at \$5.00 per copy. Those desiring to place orders for it may address the Oxford University Press, 114 Fifth Ave., New York.

**Phytohormones.**—A new volume in the *Experimental Biology Monographs* series of the Macmillan Co. is an extremely interesting summary of the work thus far accomplished in the study of plant hormones. The authors are Dr. F. W. WENT and Dr. KENNETH V. THIMANN.

The introductory chapter outlines the task of the authors, defines the hormone as a substance which, formed in one part of an organism, is transported to some other part, where it influences a specific physiological process. Earlier reviews are mentioned briefly.

The succeeding chapters deal with the development of the hormone concept; the technique of auxin determinations; formation and occurrence of auxins; the relation between auxin and growth; auxin transport and polarity; chemistry of the auxins; mechanism of the action; growth of roots; tropisms; root formation; bud inhibitions; other activities of auxins; and general conclusions.

The authors have summarized the phenomena of hormone action very succinctly, for with a lengthy bibliography, and with subject and author indexes, the book runs to only 294 pages. Although it appears only a year later than the BOYSEN-JENSEN translation by AVERY and BURKHOLDER, the authors feel that this new monograph aims more at an analysis of the problems, and the integration of the accumulated material, than has any previous summary. It is difficult to do this adequately with so many new contributions coming out each year, and with the controversial points not all settled.

The final chapters will be very helpful to students who wish to grasp quickly the general implications of the vast plant hormone literature now on record.

Every student of plant physiology should have a copy of this work, which is a timely and valuable digest of this field of research. The book is quoted at \$4.00 per copy by the publishers. Orders may be sent to the Macmillan Co., 60 Fifth Ave., New York.

**Potash Deficiency Symptoms.**—Through the cooperation of the German, French, and American potash industries, a valuable and unique summary of potash deficiency symptoms has been prepared by OSCAR ECKSTEIN, ALBERT BRUNO, and J. W. TURRENTINE, with collaboration by G. A. COWIE and G. N. HOFFER. The book is profusely illustrated, with 41 text figures, and 54 beautiful colored plates depicting the deficiency symptoms in natural colors. There is also a colored chart showing the amounts of plant foods removed from the soil by agricultural and horticultural crop plants. Another feature of the work that will meet universal approval is the fact that the information, including the index, is printed in three languages, German, French, and English.

Following the introduction, the first part of the book deals with general symptoms of potash deficiency. This includes the effects on the leaf, root system, blossom and fruit, changes in external appearance, and modifications of the inner structures; the secondary effects of potash deficiency, especially the lowered resistance to diseases, insect pests, and untoward climatic conditions; the market value of crops in relation to potash deficiency; and the pathology of potash deficiency.

The second part deals with the specific effects on various cultivated crops. HOFFER contributes the section on maize and other cereals, and COWIE the section on fruit trees. A brief reference to deficiency symptoms of grape vines concludes the text. The 54 colored plates illustrate these specific responses, and are accompanied by brief descriptions of the symptoms shown by each crop plant. The bibliography consists of 209 titles, and there is an adequate index. The price is remarkably modest, only \$2.25 per copy. It should find a welcome place in every plant physiologist's private library. Distribution is through B. Westermann and Co., Inc., 24 West 48th St., New York.

**Pathology of the Plant Cell.**—The second volume of ERNST KÜSTER's *Pathologie der Pflanzenzelle* has appeared as volume 13 of the *Protoplasma Monographien*, published by Gebrüder Borntraeger, W 35 Koester Ufer 17, Berlin. This work considers particularly the pathology of the plastids. The two chapters deal with modifications in form, and modifications in structure, of the various plastids of plant cells. Mechanical form modifications, abnormal growth forms, deformities from capillary contraction and expansion,

abnormal divisions, and reductions in size by loss of substance are detailed in the first chapter. The second chapter takes up important structural modifications of the plastids in connection with starch storage and the presence of active pyrenoids; agglutination phenomena; swelling and vacuolization; lipophaneroze (fatty degeneration); water loss; and necrotic changes. An appendix considers pigment breakdown, and the division of the chloroplasts. More than 200 titles are cited in the bibliography; and subject and author indexes conclude the work. It is a valuable and worthy companion to KÜSTER's original monograph in the same series published about eight years ago. The price of the book bound in red cloth is RM 16. The publishers will give prompt attention to orders placed with them.

**Mitogenetic Radiation.**—Under the auspices of the Committee on Radiation of the National Research Council, ALEXANDER HOLLAENDER and WALTER D. CLAUS have made an experimental study of the problem of mitogenetic radiation. Their report is embodied in Bulletin no. 100 of the National Research Council. The work was done at the University of Wisconsin under the supervision of Dr. B. M. DUGGAR, which is a sufficient guarantee that the work was carefully planned and executed.

The results are negative. No such radiations have been detected in connection with the activity of typical biological "mitogenetic senders." The work of GURWITSCH and his followers in Europe remains entirely unconfirmed. One cannot accept the idea that these typical mitogenetic ray sources, grown in Europe, possess the property of radiation, and grown in America lack this radiation capacity. It would also be very difficult to understand how so many people could be led astray in European laboratories if mitogenetic radiations prove to be nothing but a myth. While the authors do not admit that their report is necessarily the "swan song" of mitogenetic research in the United States, it may turn out to be just that. There would seem small excuse for additional attempts to find them unless unequivocal evidence can be supplied that the methods here used are at fault, or that the investigators have not had the requisite skill to detect the radiations. The reviewer doubts that such evidence will be forthcoming.

This Bulletin no. 100 may be purchased from the National Research Council at \$1.00 per copy.

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